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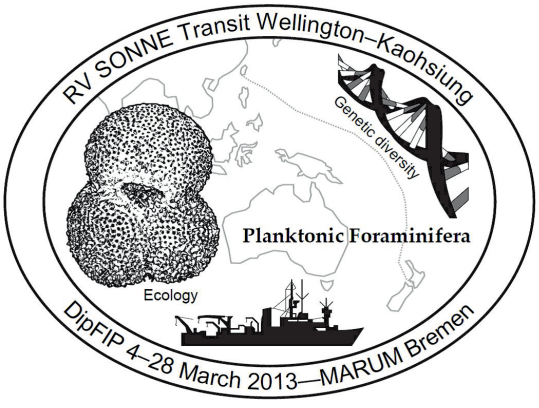
Kucera, M., Morard, R., Siccha, M., Weiner, A., Weinkauf, M.

Cruise report of RV Sonne Cruise SO226-3
DipFIP - The extent and structure of cryptic diversity in morphospecies of planktonic Foraminifera of the Indopacific Warm Pool
Wellington – Kaohsiung, 04.03.2013 - 28.03.2013



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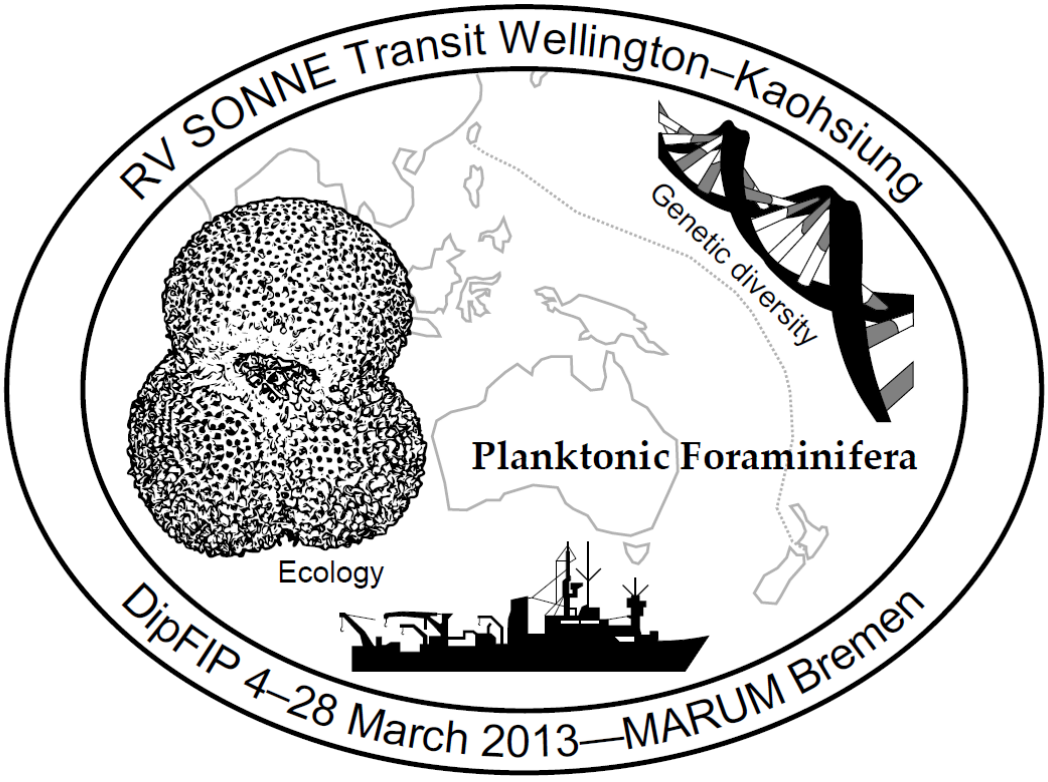
Cruise Report

DipFIP

The extent and structure of cryptic diversity in morphospecies of planktonic Foraminifera of the Indopacific Warm Pool

RV SONNE Cruise SO226-3



Wellington (04.03.2013) – Kaohsiung (28.03.2013)

Kucera, M., Morard, R., Siccha, M., Weiner, A., Weinkauf, M.

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Acknowledgements

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1. Participants

Cruise SO226-3, March 4, 2013 – March 28, 2013
Wellington (New Zealand) – Kaohsiung (Taiwan)

Scientific Party

Name	Task	Institute
Kucera, Michal	Chief Scientist, Taxonomy	MARUM
Morard, Raphael	Plankton filtration	MARUM
Siccha, Michael	Hydrography and Phytoplankton	MARUM
Weiner, Agnes	Plankton net sampling	MARUM
Weinkauf, Manuel	Plankton culturing	MARUM

Institute: MARUM – Zentrum für marine Umweltwissenschaften
Universität Bremen
Leobener Strasse
28359 Bremen
Germany

Fig. 1.1 Scientific Party of the Expedition SO226-3 crossing the Equator.

Cruise SO226-3, March 4, 2013 – March 28, 2013
Wellington (New Zealand) – Kaohsiung (Taiwan)

Name	Rank
Meyer, Oliver	Master
Korte, Detlef	Chief Officer
Goebel, Jens Christian	2. Officer
Henning, Tim	2. Officer
Dr. Bauer, Bodo	Ship's Doctor
Leppin, Joerg	Chief System Manager
Borchert, Wolfgang	System Manager
Guzman-Navarrete, Werner	Chief Engineer
Klinder, Klaus-Dieter	2. Engineer
Genschow, Steffen	2. Engineer
Beyer, Thomas	Electrician
Blohm, Volker	Fitter
Kuderski, Jens	MPC / Motorman
Krawczak, Ryszard	MPC / Motorman
Tiemann, Frank	Chief Cook
Matter, Sebastian	2. Cook
Garnitz, Andre	2. Cook
Schmandke, Harald	Chief Steward
Steep, Maik	2. Steward
Schrapel, Andreas	Boatswain
Ross, Reno	MPC / A.B
Fricke, Ingo	A.B.
Kraft, Juergen	A.B.
Staengl, Guenter	MPC / A.B.
Kuhn, Benedict	Trainee
Thimm, Sebastian Uwe	Trainee
Altendorf, Denis	A.B.
Barkow, Michael	A.B.

2. Research Programme

The extent and structure of cryptic diversity in morphospecies of planktonic Foraminifera of the Indopacific Warm Pool (DipFIP)

Planktonic foraminifera are free-living unicellular heterotrophic organisms. They form a relatively small portion of the oceanic plankton biomass, but their calcite shells accumulate in huge quantities on the sea floor and form a significant constituent of deep-sea sediment. Their high abundance in marine sediments and seemingly well resolved taxonomy made planktonic foraminifera one of the most important tools in biostratigraphy and paleoclimatology. Today, the vast majority of methods for determination of surface ocean properties in the past are based on analyses of the chemical composition of foraminiferal shells or their shell morphology and assemblage composition (e.g. Kucera 2007). Foraminifera-based proxies for past ocean properties are empirically derived and require species-specific calibrations. This is due to physiological differences among species as well as their different habitat preferences recording conditions at different depths in the water column during the season of their maximum production (e.g. Hemleben et al. 1989). Therefore, correct assessment of species taxonomy, ecology and biogeography in planktonic foraminifera is essential not only for biological and ecological studies but also for geochemical reconstructions of past environments.

The classification of planktonic foraminifera is based entirely on the morphology of their shells. In this way, the taxonomies of modern and fossil representatives of this group are mutually entirely consistent, allowing the transfer of proxy calibration results from the modern ocean on fossil material. It has long been recognised that species of planktonic foraminifera are highly variable in their morphology, but the significance of these phenomena has not been understood, until the first molecular genetic investigations pointed out the existence of multiple genetically distinct lineages within morphological species (Darling and Wade 2008). The discovery of distinct genetic types within many planktonic foraminiferal morphospecies implied that the taxonomy of this group had to be revised. Existing evidence suggests that many of the cryptic genotypes represent distinct biological species and, in some cases, reticent morphological characters have been identified that could be linked to these genotypes (e.g., Morard et al. 2009; Aurahs et al., 2011).

The extent and spatial structure of such “cryptic” genetic diversity is only known in a limited number of species. To date, only 26 out of the 47 morphospecies of planktonic foraminifera are known by their SSU rDNA sequence (Aurahs et al., 2009a). For multiple species, the sampling density is now extensive enough to allow first hypotheses about spatial genotype segregation and habitat preferences of their constituent genetic types. In many cases, the habitat of the morphological species has been shown to be partitioned among the cryptic genetic types, which show spatially or ecologically more restricted distributions (de Vargas et al., 1999; Weiner et al., 2012). These distributions have been correlated with water masses and oceanographic variables, but Aurahs et al. (2009b) also identified biotic interaction as a potential factor controlling the distribution of the genetic types. Whereas the first model allows a parameterisation of the habitat space of the species by environmental variables, the second model (also known as niche partitioning) leads to spatial patterns reflecting historical contingency, species interactions and habitat stability.

Since the traditional morphological species concept of planktonic foraminifera is not adequate to capture processes at the level of biological species (André et al., 2013), an approach combining classical sampling and habitat characterisation of morphospecies with molecular characterisation of individual taxa is required. In this way, the partitioning of the habitat of individual morphological species among their constituent genetic types can be characterised and used to constrain paleoceanographical and ecological interpretations based on morphological species.

In this respect, the cruise track of the expedition SO226-3 offers an excellent opportunity to investigate the habitat partitioning among genetic types of planktonic foraminifera throughout the entire tropical and subtropical western Pacific. This region has never been sampled in this extent before; previous genetic surveys in the Pacific have been limited by sample size (Ujiie and Lipps, 2009) or they reported on individual species (Ujiie et al., 2012). The western Pacific transect is of interest not only because it represent an undersampled region. First, it allows the comparison across a strong trophic gradient, which is decoupled from temperature. Second, it provides the opportunity to investigate diversity within the unique water mass of the Western Pacific Warm Pool and third, it allows us to constrain the occurrence of genetic types in the upstream region of the Indonesian Throughflow, which is a critical pathway for gene flow between the Pacific and Indian Oceans.

Benefiting from a newly established DNA extraction method, we are now able to combine during one cruise classical plankton analysis in stratified samples with the possibility to obtain DNA from all preserved specimens. This approach will be applied using the standard 9-depth-level sampling scheme of the upper 700 m of the water column throughout the southern and northern subtropical waters and the equatorial circulation system of the western Pacific. We specifically aim to determine whether the gradients of diversity in morphological species as observed across this region in surface sediments (Rutherford et al., 1999) is mirrored at the genetic level of species identification. We also aim to constrain the vertical habitat of key morphological species, which are frequently used for paleoceanographic reconstructions, as well as to determine the degree to which the population density and structure at morphological and genetic level can be predicted by water mass properties.

The cruise also provides an opportunity to sample planktonic foraminifera species which are endemic to Indopacific warm waters and/or for which no DNA sequences have been obtained so far. An increased taxon sampling is essential to make the most of the opportunity to combine DNA-based phylogeny of the group with data from the fossil record (Aze et al., 2011). Such a comparison has high potential to deliver information on the causes of the high variability in DNA substitution rates known for this group (de Vargas et al., 1997).

Apart from a test of the feasibility to culture planktonic foraminifera on board, as has been recently reported by Manno et al. (2012), we also aim for the first time to collect bulk plankton samples for a next generation sequencing (NGS) approach. The current single-cell DNA analysis approach is well established and powerful, but time consuming and sensitive to DNA degradation during collection. These two factors limit its throughput, making its use challenging for questions where many samples and individuals have to be analysed. After the two decades of single-cell classical sequencing and methodological advances in species identification from sequence divergence (Göker et al., 2010) it is now possible to attempt to apply NGS to assess

the structure and spatiotemporal dynamics of the diversity in planktonic foraminifera on a scale not accessible before. The material collected during this cruise will be used to test the fidelity (rate of taxa identification) of the approach and establish the scaling between NGS sequence abundance and population structure by parallel analysis of simultaneous samples by morphological taxonomy and classical sequencing. The material will be collected by filtration of plankton net samples, where the population of planktonic foraminifera present prior to analysis will be approximated from species counts in parallel net hauls from the same depth at the same stations. In addition to the analysis of planktonic foraminifera DNA, the filter samples have the potential to deliver data on the rest of the plankton community, including potential prey and symbionts of the resident planktonic foraminifera.

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3. Cruise Narrative

Having embarked the ship in Wellington on 3.3.2013 at noon, the scientific party used the rest of the day to mobilise equipment and install the laboratories. On Monday 4.3.2013 at 10:30 local time, with fair weather and calm sea, the ship has left the harbour of Wellington heading NW for the Tasman Sea. Although the weather remained good, heavier swell was encountered in the night. In the morning of 5.3.2013, the edge of the Zealandia shelf has been crossed and the first station was taken over the continental slope in the Tasman Sea. The sampling programme, which will be with small modifications repeated at every station, consisted of a CTD cast to 1000 m, followed by two plankton net hauls to 700 m and 100 m, with standard sampling intervals. Both the ship CTD and our plankton net are working fine, although a tear in one of the nets has been discovered. The fluorescence probe yielded a dataset which appears unlikely to be correct. The CTD cast revealed a shallow mixed layer of about 40 m and a low population density of foraminifera, showing a typical temperate assemblage. Heavy swell continued whilst the ship was heading North.

The second station was taken close to the northernmost tip of New Zealand in the morning on 6.3.2013, yielding a rich temperate assemblage of planktonic foraminifera. At this station and all following stations, it was decided to sample with the CTD only the top 800 m of the water column. A third plankton net haul to 700 m was taken to cover the entire water column and test the filtration apparatus for use with parallel samples. The EEZ of New Zealand was crossed on 7.3.2013 at 6 am local time, the EEZ of New Caledonia has been reached on 8.3.2013 at 4 pm local time. Three stations were taken on the way, always at the same time of the day. The CTD data indicated subtropical conditions with thermally mixed layer reaching to 100 m, followed by a shallow and thick thermocline.

On 8.3.2013 the weather report showed a tropical storm north of New Caledonia, which was predicted to develop slowly towards the south. The associated winds and swell were considered too high to continue in the planned direction and the cruise track has been modified to pass further east. The last station in the EEZ of New Caledonia has been taken on 9.3.2013 and the next two days were spent by transiting the EEZ of Vanuatu, where no stations were taken due to lacking permission. In the wake of the large tropical storm, the weather deteriorated with heavy swell, clouds and rain.

The EEZ of the Salomon Islands has been reached on 12.3.2013 and the first station in the morning on that day revealed a water column structure indicative of a complex equatorial circulation system, including strong subsurface salinity maximum and a steep thermocline suggestive of up doming of deep waters. After five unsuccessful attempts, it was decided not to deploy the fluorescence probe in the water any more but to confront it with water from CTD-Rosette bottles instead. In the afternoon, the Duff Islands have been sighted. The last station in the EEZ of the Salomon Islands was taken on 13.3.2013, including one additional net haul to 700 m for filtration. The thermal mixed layer reaches almost to 200 m, the topmost 20 m harbour a particularly rich population of planktonic foraminifera. After a brief spell on high seas, the modified cruise track took us through the EEZ of Papua New Guinea on 14.3.2013, where no station could be taken due to lacking permission.

The cruise continued heading northwest, the sea has calmed and the clouds lifted in the afternoon. The next station was taken on 15.3.2013 at 10:30 local time as soon as the EEZ of Papua New Guinea was abandoned. On 16.3.2013 at 2 am local time, the Equator was crossed. Operating between high seas and the EEZ of Micronesia, the ship follows a straight track heading away from the equatorial waters above the Ontong Java Plateau towards the subtropical waters of the deep Philippine Sea. No station was taken on 16.3.2013, because the net haul from the previous day was too rich and its processing took another day.

The next three stations were taken between 17.3.2013 and 19.3.2013, all still recording the complex equatorial circulation system. On 19.3.2013, a third net haul for filtration was taken. The samples were so rich that on 20.3.2013 only a CTD has been deployed and the counting and picking of foraminifera continued the entire day. The Island arch of the South Marianas has been crossed in the night to 21.3.2013 and the next station was taken in the morning, now in the deep, cooler and oligotrophic waters of the Philippine Sea. The assemblage of foraminifera was still tropical, with lower density, but conspicuously deeper penetration in the water column, which displayed the lowest phytoplankton pigment concentration measured so far. The weather turned cloudy again, with slightly higher swell.

Heading NNW for our final destination, the last three stations were taken in international waters, before reaching the EEZ of the Philippines on 24.3.2013. The three stations revealed a typical subtropical water layering with a thick mixed layer and a clearly developed oxygen minimum. At all three stations, three net hauls have been taken, with one 700 m haul per station being used entirely for filtration. The clouds have broken again on 21.3.2013 and good weather with moderate swell has accompanied the ship on its way to Taiwan.

The last two days of the cruise the ship steamed through the EEZ of the Philippines and Taiwan, where no permissions were available. The transit has been used to demobilise equipment and consolidate cruise data. The ship has reached the port of Kaohsiung, Taiwan, on 27.3.2013 at 4 pm local time. The demobilisation was completed on 28.3.2013 and the first part of the scientific party disembarked the ship. On 29.3.2013, samples for genetic studies have been handed over to courier for transport on dry ice back to Bremen and the remaining scientists disembarked the ship at 1 pm local time, marking the end of the expedition SO226-3.

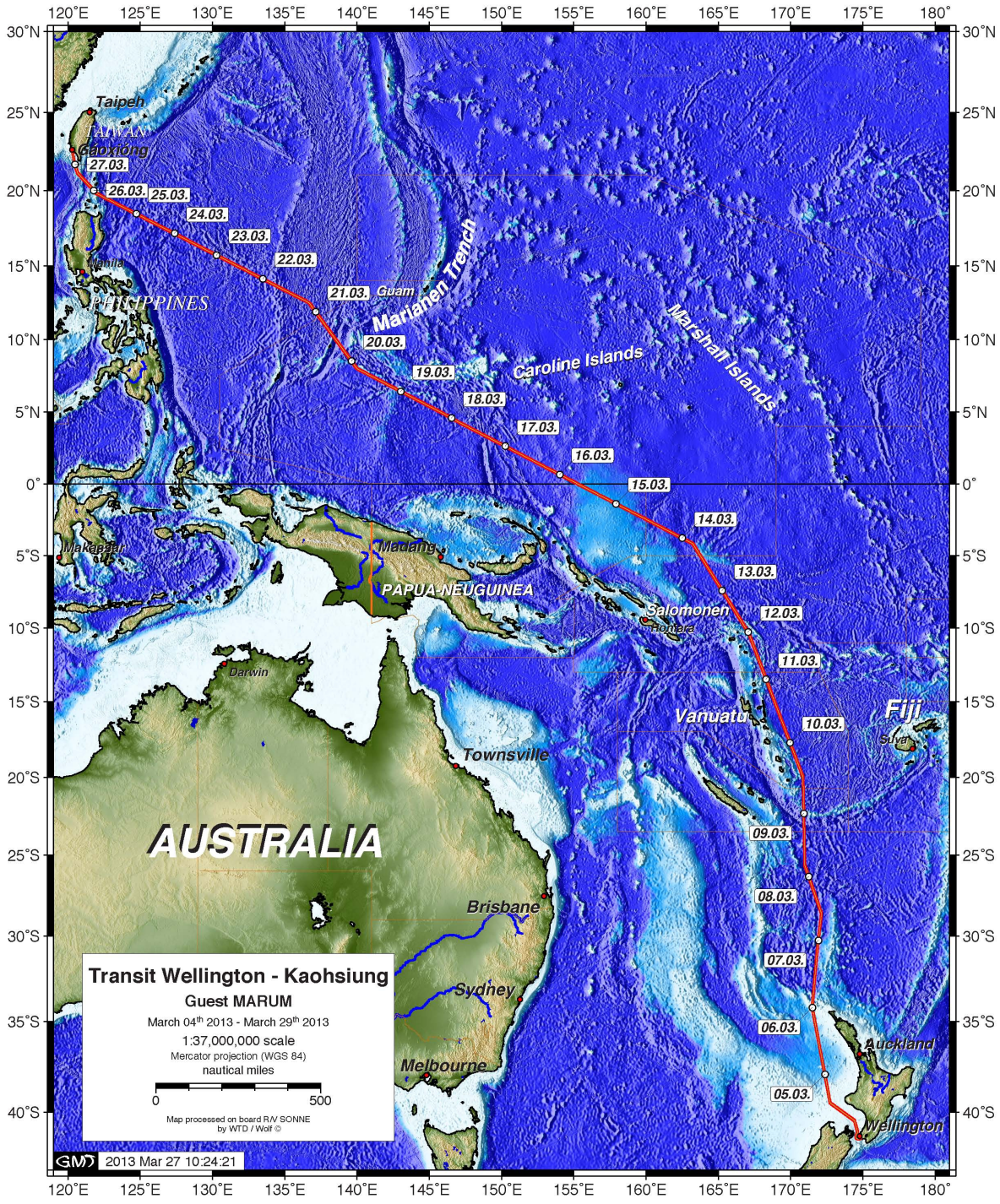


Fig. 3.1 Cruise track of the Expedition SO226-3 (DipFIP).

4. CTD Profiling and Phytoplankton Analysis

The onboard *Seabird SBE 9 plus* CTD (Conductivity-Temperature-Depth) was used to obtain measurements of temperature, salinity and dissolved oxygen in the water column. CTD deployments were performed at every station to a depth of 800 m (exception being Station 1 with a 1000 m deployment depth). The CTD is equipped with a SBE carousel water sampler with 24 10l Niskin bottles. Starting from Station 6 we used water from the CTD water sampler to measure pigment concentration *ex situ* with a fluorospectrometer. Obtained hydrographic data were binned to 1 m intervals with the available *SBE* software. Obvious outliers in the readings of the oxygen sensor close to the sea surface have been manually removed. For all parameters and figures in this report, downcast readings have been used.

We used a submersible fluorospectrometer (*bbe Moldaenke*) to determine algae pigment concentrations in the water column. The instrument uses six LEDs with different excitation wavelengths to determine concentrations of five algae classes (green algae, bluegreen algae, diatoms, cryptophytes and yellow substances) via their fluorescence fingerprints. After initial failures of the fluorospectrometer to take in situ measurements while mounted on the multinet, we switched to taking measurements in the lab on water collected with the Niskin bottles of the CTD. Beginning with station SO226-3-6 we sampled 24 depths per station. The sampling depths (475 m to 325 m in 50 m intervals, 290 m to 110 m in 20 m intervals and 95 m to 5 m in 10 m intervals) were chosen to allow useful binning of the data for comparison with the foraminifera assemblage data and to obtain a higher resolution in shallower depths, where most of the phytoplankton was expected to occur.

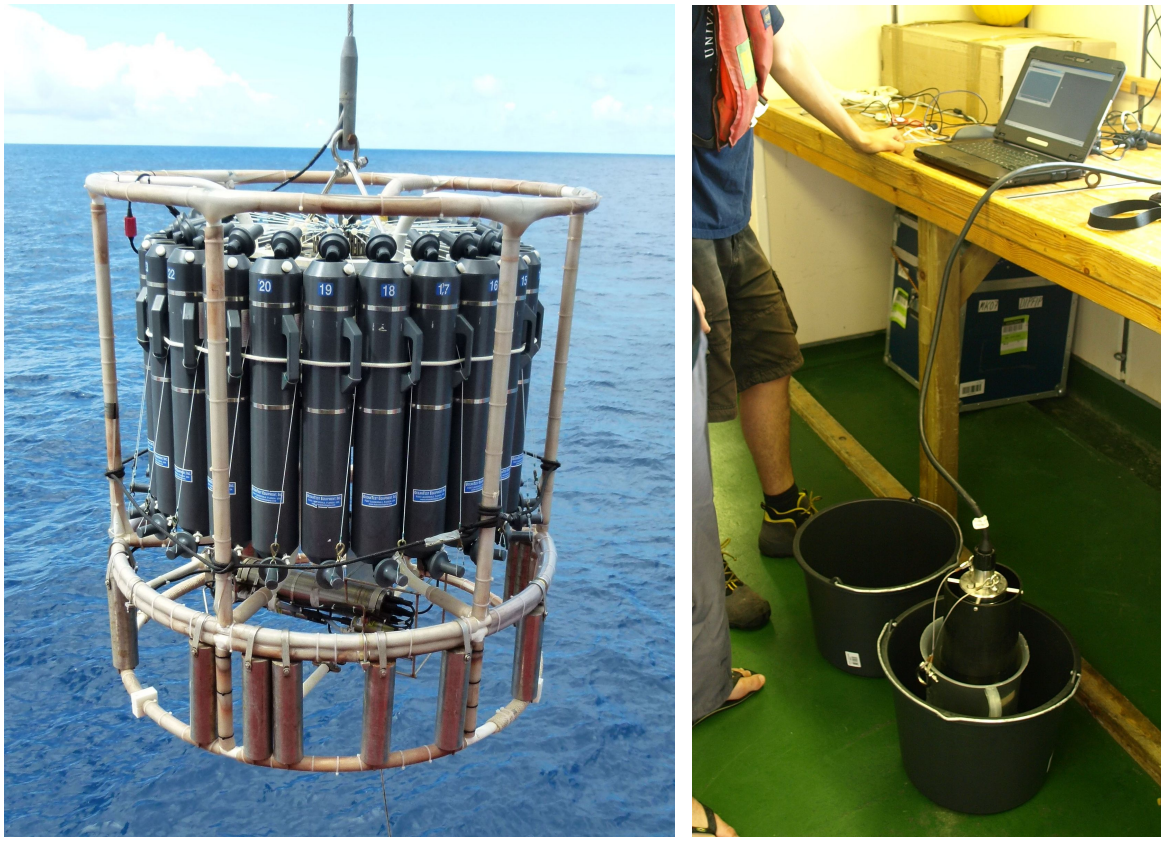


Fig. 4.1 CTD *Seabird SBE 9 plus* with water sampler carousel and the *bbe Moldaenke Fluoroprobe* Fluorospectrometer during measurements of CTD water samples onboard during SO226-3.

Starting from the deepest level, approximately five litres of seawater were taken from each Niskin bottle in a clear plastic container. The container was placed into a black plastic bucket to minimise daylight exposure and the fluorospectrometer was submerged in seawater whilst connected to a computer. Measurements were taken for a period of about 90 seconds, resulting in 30 readings per sample, which were then averaged. The consistency of the measured values was high, although we noted a deterioration of the signal (high standard deviation of the measurements) at low concentrations.

In order to guide the sampling, MODIS satellite images of surface chlorophyll concentration have been taken daily (whenever available) and merged into a composite additive overlay (Fig. 4.2).

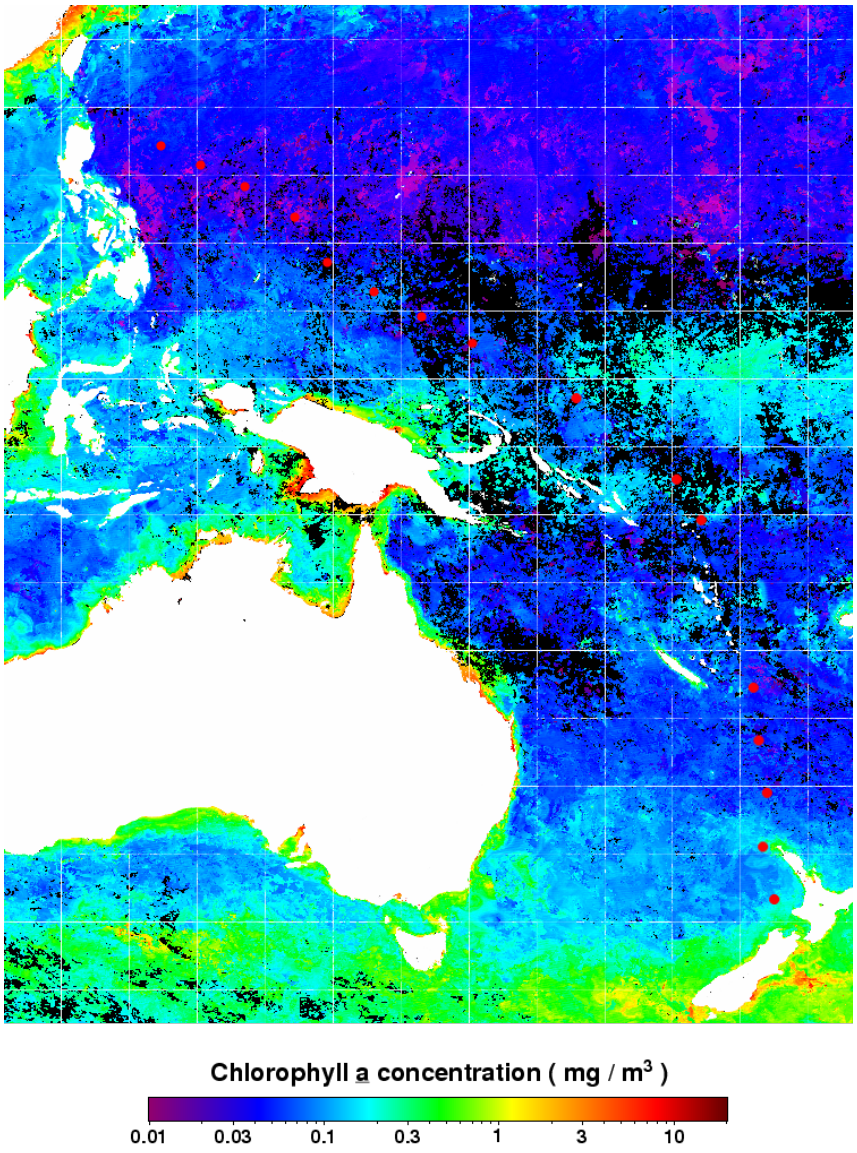


Fig. 4.2 Composite image (March 3rd to March 26th) of daily Aqua MODIS chlorophyll concentration images obtained from the OceanColor website.

5. Plankton Sampling

During the transit, planktonic foraminifera were collected for molecular genetic studies and habitat characterisation using a multiple closing plankton net (HydroBios, Kiel) with 50 × 50 cm opening, 100 µm mesh and five net bags, which allows stratified vertical sampling in five depth intervals per haul. The nets were taken at the same time of the day, between 9 and 11 am local time. A total of 15 stations were sampled. At nine stations two nets and at six stations three nets were taken (Table 5.1). The sampling depth was the same for all stations with a maximum depth of 700 m for the deep net and 100 m for the shallow net. Two different sets of depth intervals were applied (100–80 m, 80–60 m, 60–40 m, 40–20 m, 20–0 m and 700–500 m, 500–300 m, 300–200 m, 200–100 m, 100–0 m). This sampling scheme provides a nine-level resolution of the water column with one spare bulk sample for the 100–0 m level, which could be used for filtration.

In all deployments, the determination of closing depths was based on depth readings from the pressure sensor on the net. In all cases, strict vertical hauls have been carried out, allowing an approximation of the filtered volume by multiplying the net opening area with the length of haul per net bag. The angle of the net deployment was checked by comparing pressure reading from the net with the rope length. The latter never exceeded the depth reading by more than 10%. Slacking and hoisting was done at 0.5 m/s. After each haul the net bags were washed with sea water, inspected for damage, and the net cups were rinsed and cleaned with filtered sea water. A tear was discovered in one of the nets at the first station. The net has been subsequently exchanged, but the data from one or two depth intervals, particularly in the shallowest sample from that station, may have been affected.

Fig. 5.1 Slacking of the plankton net (left) and changing the net cups (right) during SO226-3.

Tab. 5.1 Summary of plankton net samples taken during SO226-3, including the number of foraminifera picked from each net haul and the number of depth intervals taken for filtration (NFI) and the number of filters used per net. Blank samples for filtration have been taken by filtering bulk residues after picking of foraminifera from all nine depth intervals at three stations.

Net	Date	Station	Depth	Foraminifera	Filtered depth	NFI	Filters
K273	2013-03-04	226-3-1	700 m	128	0-100 m	1	1
K274	2013-03-04	226-3-1	100 m	34			
K275	2013-03-05	226-3-2	700 m		0-700 m	5	8
K276	2013-03-05	226-3-2	700 m	2610	0-100 m	1	3
K277	2013-03-05	226-3-2	100 m	1825			
K278	2013-03-06	226-3-3	700 m	810	0-100 m	1	3
K279	2013-03-06	226-3-3	100 m	483			
K280	2013-03-07	226-3-4	700 m	254	0-100 m	1	1
K281	2013-03-07	226-3-4	100 m	997			
K282	2013-03-08	226-3-5	700 m	342	0-100 m	1	3
K283	2013-03-08	226-3-5	100 m	579			
K284	2013-03-11	226-3-6	700 m	1028	0-100 m	1	3
K285	2013-03-11	226-3-6	100 m	2966			
K286	2013-03-12	226-3-7	700 m		0-700 m	5	13
K287	2013-03-12	226-3-7	700 m	854	0-100 m	1	4
K288	2013-03-12	226-3-7	100 m	3990			
K289	2013-03-14	226-3-8	700 m	2084	0-100 m	1	1
K290	2013-03-14	226-3-8	100 m	2792			
K291	2013-03-16	226-3-9	700 m	1280	0-100 m	1	2
K292	2013-03-16	226-3-9	100 m	1550			
K293	2013-03-17	226-3-10	700 m	599	0-100 m	1	3
K294	2013-03-17	226-3-10	100 m	3638			
K295	2013-03-18	226-3-11	700 m		0-700 m	5	7
K296	2013-03-18	226-3-11	700 m	2453	0-100 m	1	3
K297	2013-03-18	226-3-11	100 m	6312			
K298	2013-03-20	226-3-13	700 m	1930	0-100 m	1	1
K299	2013-03-20	226-3-13	100 m	1333			
Blank	2013-03-20	226-3-13			0-700 m	1	1
K300	2013-03-21	226-3-14	700 m		0-700 m	5	5
K301	2013-03-21	226-3-14	700 m	693	0-100 m	1	1
K302	2013-03-21	226-3-14	100 m	822			
Blank	2013-03-21	226-3-14			0-700 m	1	1
K303	2013-03-22	226-3-15	700 m		0-700 m	5	5
K304	2013-03-22	226-3-15	700 m	269	0-100 m	1	1
K305	2013-03-22	226-3-15	100 m	772			
Blank	2013-03-22	226-3-15			0-700 m	1	1
K306	2013-03-23	226-3-16	700 m		0-700 m	5	5
K307	2013-03-23	226-3-16	700 m	262	0-100 m	1	1
K308	2013-03-23	226-3-16	100 m	1099			
Total:				**44788**		**48**	**77**

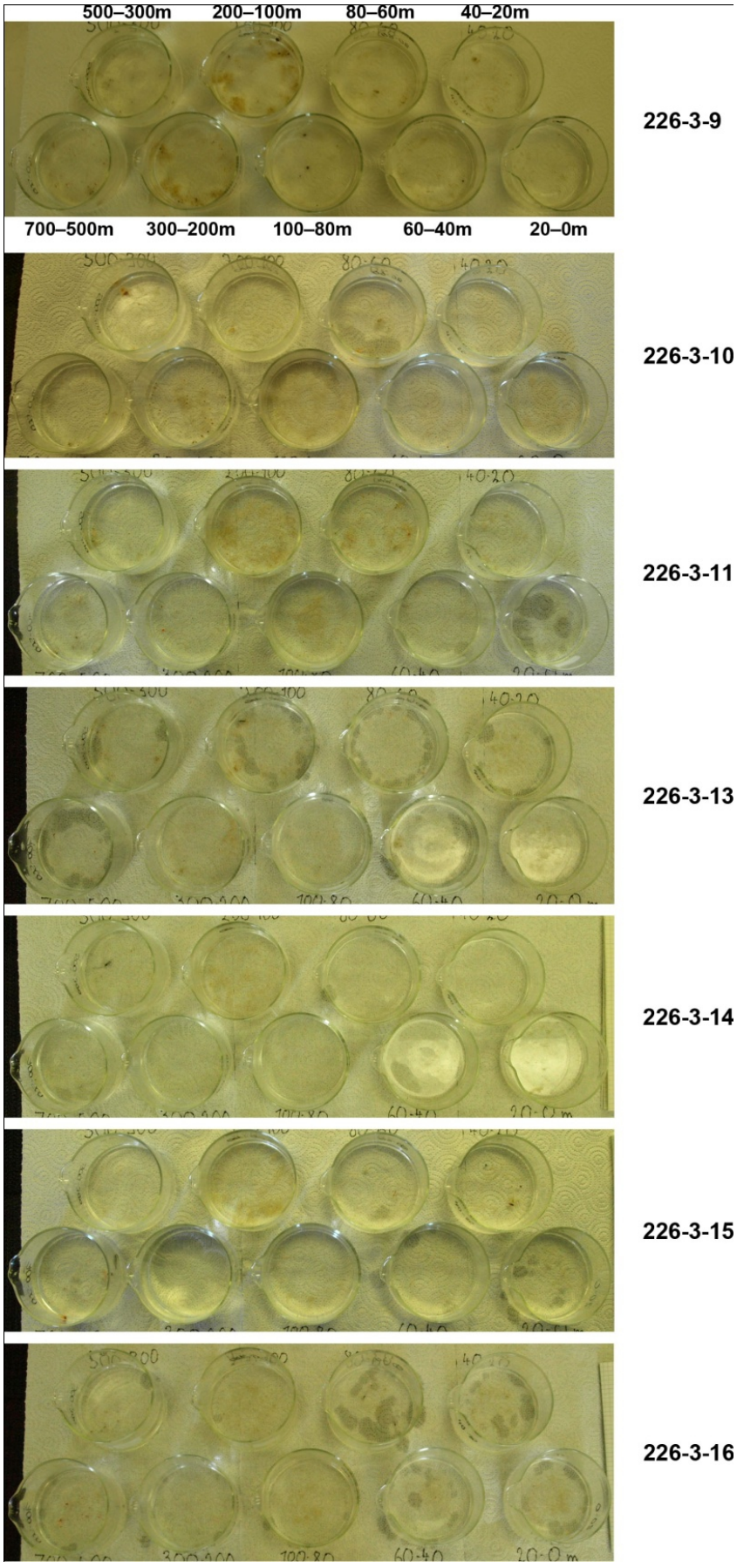


Fig. 5.2 Plankton net samples transferred into glass dishes for picking. The order of the depth intervals is the same for all samples as shown for station SO226-3-9.

For net hauls which were used to collect foraminifera, the plankton residues were first transferred from the net cups into glass dishes (Fig. 5.2). The cloth of the cups was carefully rinsed with filtered sea water to remove any adhering plankton. The foraminifera were then picked out of the residues under a stereomicroscope (Zeiss Stemi 2000), cleaned with a fine brush and the individuals from one depth interval were all placed together on a cardboard slide (or multiple slides where too many specimens have been encountered). The numbers of empty shells (entirely free of cytoplasm or containing only small isolated patches of cytoplasm distributed discontinuously throughout the shell) and specimens bearing cytoplasm were recorded separately in all samples. Census counts were carried out wherever time allowed, all by the same person (MK), following the taxonomy by Bé (1977), Parker (1962) and Hemleben et al. (1989). Raw counts were converted to concentrations per unit volume by dividing specimen counts in each net interval by the sampled volume of water. After counting, the slides were frozen at −80 °C to prevent DNA degradation until further processing in the laboratory.

For the picking of foraminifera on board, samples were randomly assigned to one of the five scientists, the name of the person picking each depth interval recorded, and the results in adjacent samples were compared. In this respect, the similarity among the results by all five persons involved in the picking was remarkably high and the counts can thus be considered mutually consistent. Depending on the amount of material in the sample and the degree of aggregation of plankton particles in the sample, picking was carried out either directly from the glass dishes or from purified concentrates. Using fine pipettes, brushes and needles to disintegrate aggregates, foraminifera were cleaned and transferred to the slides, where specimens with adhering plankton aggregates were further cleaned. Individual specimens were then separated on the slides to avoid mutual contamination. Under favourable conditions, the foraminifera could be concentrated in the middle of the glass dish by swirling the dish (Fig. 5.3). The concentrate was then transferred into a new dish and the procedure was repeated to purify the foraminifera fraction, which was then transferred by a pipette into a Petri dish or onto cellulose filters, from where the foraminifera could be picked faster. In any case, the entire sample has been inspected and picked, including the smallest specimens that could be recognised and manipulated.

Imaging of selected specimens (Figs. 5.4 and 5.5) was performed using either a Zeiss STEMI 2000 stereomicroscope or a Zeiss PrimoVert inverted microscope. Both systems were fitted with a camera tubus with a Canon EOS 600D DMR camera and images were saved directly to a connected notebook. For photographing with the STEMI 2000, specimens were placed in separate sea-water filled wells of a microwell-plate and photographed under sideway illumination. For the PrimoVert inverted microscope, HydroBios observation chambers were used for imaging under sideway illumination and in transmitted light. The chambers consist of a metal ring holding a plastic cylinder closed at the bottom by a cover glass. Filling the chambers completely with sea water (10 ml) and covering them with a thin plastic lid reduces reflexions due to water movement and distortions of the margins due to the water surface meniscus.

Besides fixing of specimens for DNA analysis by drying and storing at -80°C, selected individuals of rare species or species never sequenced before were placed in the GITC* buffer (Morard et al., 2009) as an alternative preservation method to

increase the probability of successful DNA extraction from these species. Specimens were described and individually transferred on a tip of a fine brush into labelled Eppendorf tubes with 50 µl of the buffer. The tubes were immediately stored at -20°C. A total of 53 individuals have been preserved in this way (Table 5.2). All material collected during the cruise, including dried slides with foraminifera, foraminifera in GITC* buffer and bulk filters samples were shipped to Bremen on dry ice after the end of the cruise.

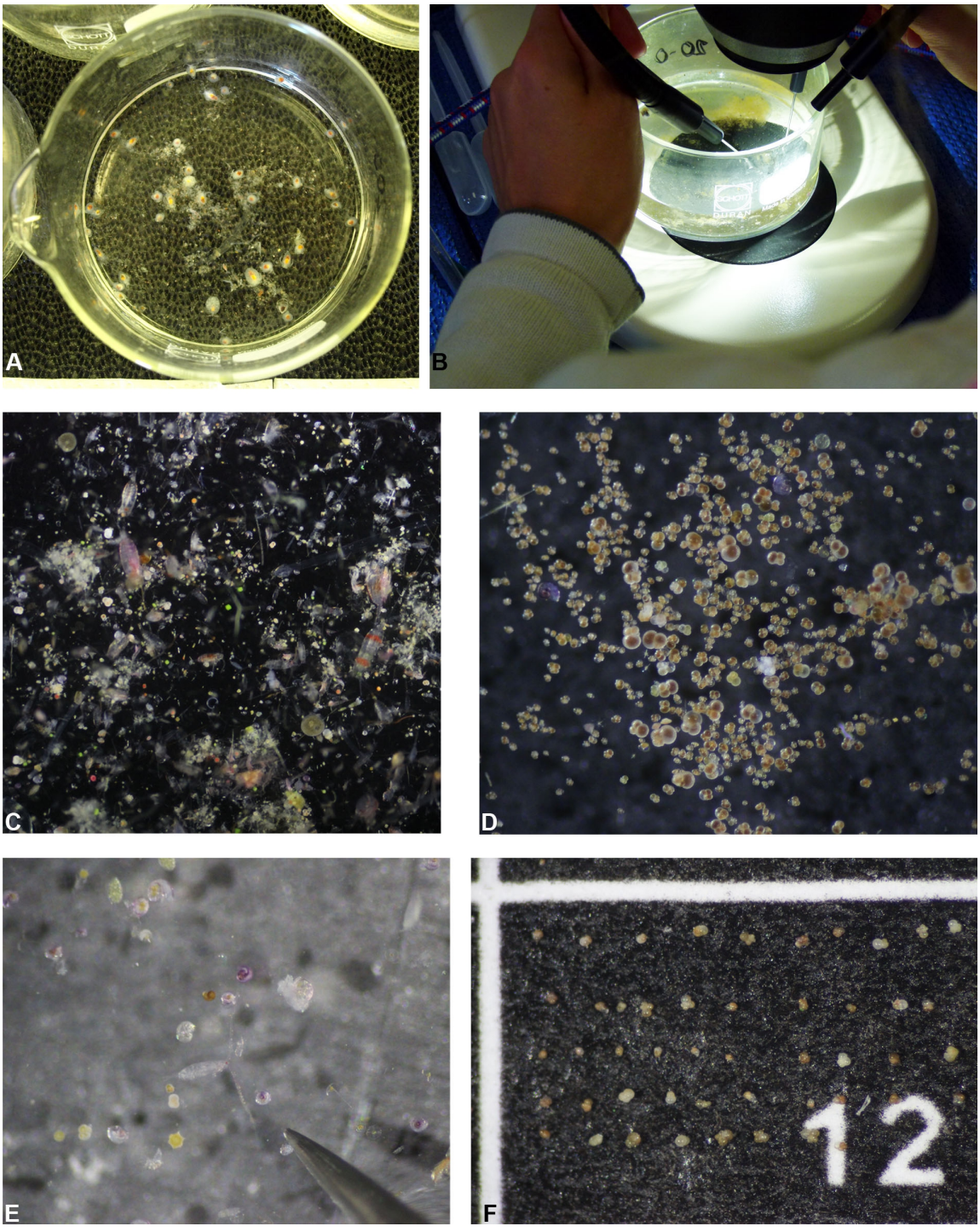


Fig 5.3 Plankton samples in a glass dish showing particle aggregates and large pteropods (A). Aggregates of algae disintegrated with picking needles (B). The foraminifera in plankton residue shown in (C) could often be concentrated in the middle of a Petri dish by swirling (D). The foraminifera were picked using needles and brushes (E), and transferred to cardboard slides and separated (F).

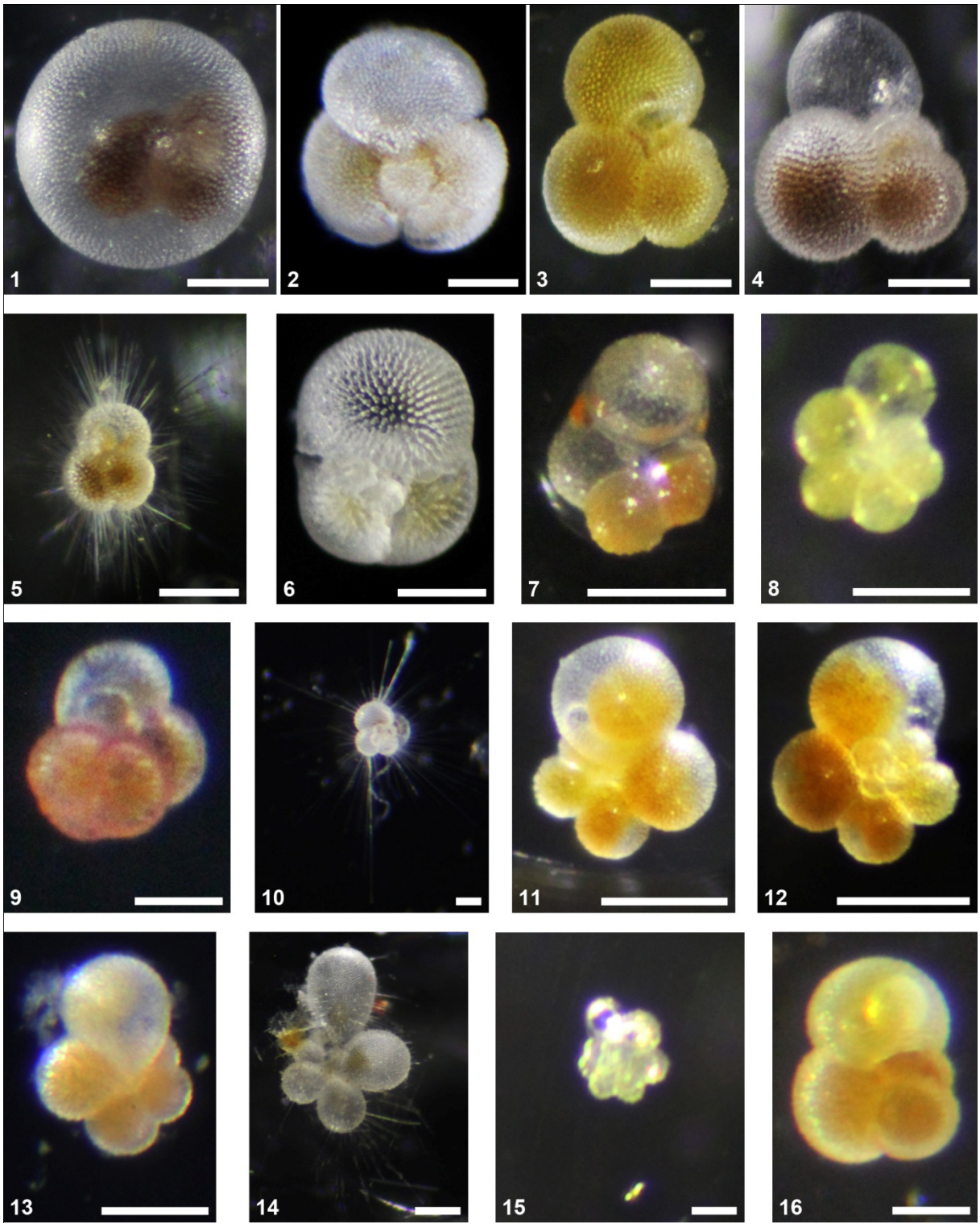


Fig. 5.4 Representative specimens of spinose (1–15) and microperforate (16) planktonic foraminifera encountered during the expedition SO226-3: (1) *Orbulina universa*, (2) *Globigerinoides conglobatus*, (3, 4) *Globigerinoides sacculifer* (with and without a sac-like terminal chamber), (5) *Globigerinoides ruber*, (6) *Sphaeroidinella dehiscens*, (7) *Beella digitata*, (8) *Turborotalita quinqueloba*, (9) *Globoturborotalita rubescens*, (10) *Globoturborotalita tenella*, (11, 12) *Globigerinella siphonifera* from umbilical and spiral side, (13) *Globigerinella calida*, (14) *Globigerinella adamsi* (or an unusually large specimen of *G. calida*), (15) *Turborotalita humilis*, (16) *Globigerinita glutinata*. Scale bar equals 50 µm in (15), 100 µm in (8–10) and (16), and 200 µm in all other cases.

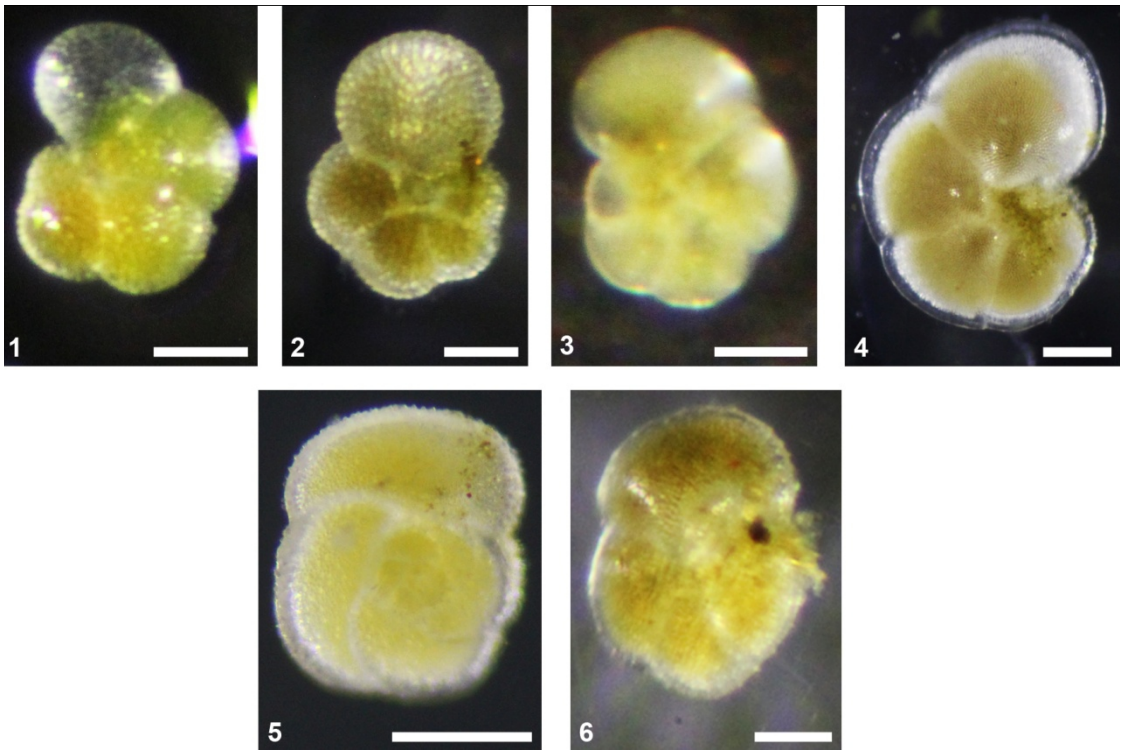


Fig. 5.5 Representative specimens of nonspinose planktonic foraminifera encountered during the expedition SO226-3: (1) *Neogloboquadrina dutertrei*, (2) *Globorotaloides hexagonus*, (3) *Globorotalia scitula*, (4) *Globorotalia menardii*, (5) *Globorotalia crassaformis*, (6) *Globorotalia theyeri*. Scale bar equals 200 µm in (4, 5) and 100 µm in all other cases.

Tab. 5.2 Overview of planktonic foraminifera preserved during the cruise SO226-3 for DNA analysis in the GITC* buffer.

Species name	**N**
Turborotalita humilis (including *T. cristata* form)	12
Globorotaloides hexagonus	9
Dentigloborotalia anfracta	4
Globorotalia crassaformis	4
Globorotalia theyeri	3
Globigerinoides conglobatus	3
Globoturborotalita tenella	2
Sphaeroidinella dehiscens	2
Streptochilus globigerus	2
Tenuitella? sp.	2
Globigerinoides ruber (*G. pyramidalis* form)	1
Globigerinella calida	1
Globigerinita minuta	1
Globigerina bulloides	1
Globorotalia menardii	1
Unidentified juvenile specimen	1
Neogloboquadrina incompta from culture	1
Globigerinoides ruber from culture	1
Globigerinoides sacculifer (*G. trilobus* form) from culture	1
Hastigerina pelagica gametogenic specimen from culture	1

In order to obtain bulk plankton samples for DNA extraction and environmental sequencing, a number of plankton net samples have been filtered on board. The filtration setup consisted of a two-level board holding 5 canisters with a capacity of 5 litres each, five gaskets to hold the filters, a pump and a vacuum bottle (Fig. 5.6). Before sample processing, the canisters were carefully rinsed with MilliQ water to avoid contamination. The gaskets were cleaned with 96% ethanol and MilliQ water. Before sample collection, the filters (8-12 µm round cellulose filters) were placed into the gaskets with sterilized tweezers and the lid was tightly screwed. Immediately after collection, the plankton samples were transferred from the net cups to the canisters trough a 1 mm sieve to remove large plankton from the sample. The net cups were carefully rinsed several times with filtered seawater to retain all plankton particles. The canisters were then placed onto the upper broad of the filtering device, connected to the gaskets and the filtration was conducted using the vacuum pump.

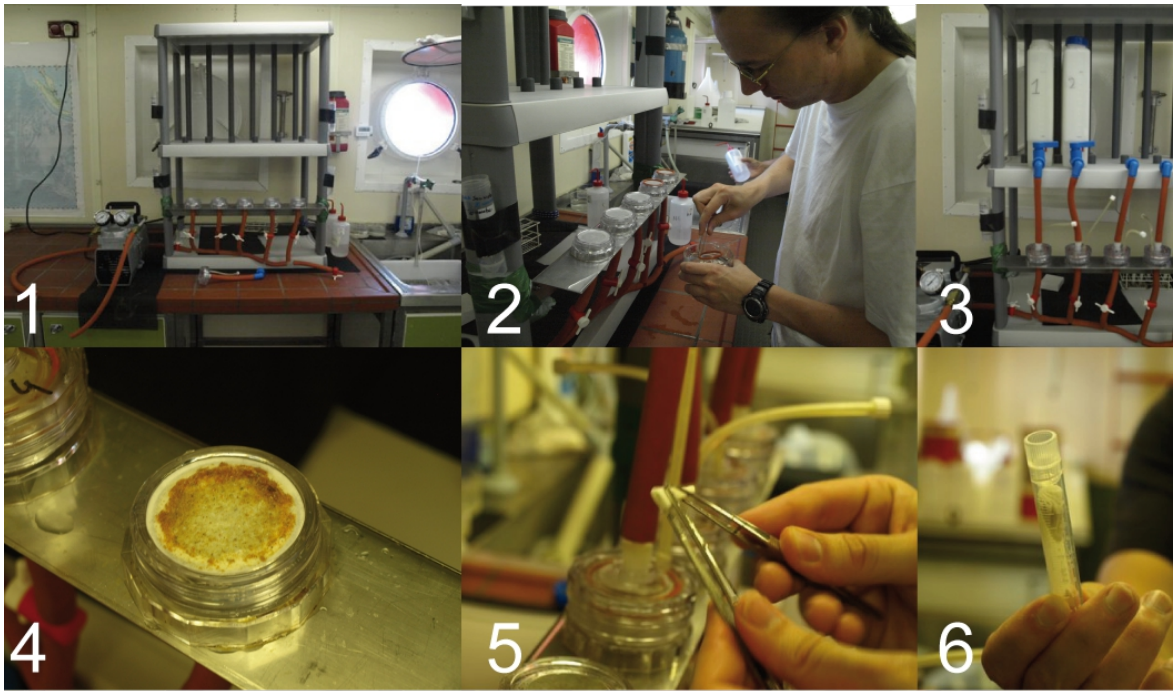


Fig. 5.6 The filtration apparatus and its application on plankton samples during the expedition SO226-3. (1) the filtration apparatus without the water canisters and gaskets, (2) placing filters on the gaskets, (3) the apparatus with two canisters connected to the gaskets, (4) the plankton concentrate after filtration, (5) folding of the filter with plankton concentrate and (6) storing of the filters in plastic tubes.

For 36 out of the 48 samples filtered during the cruise, a single filter has been sufficient to process the complete sample. In this case, the canister has been removed for cleaning and the lid of the gasket unscrewed. The filter with the plankton concentrate was folded several times using sterilized tweezers and then placed into a 5 ml tube (Fig. 5.6). The tube was then placed in the -80°C freezer. In case of incomplete processing of the sample because of clogging of the filters, the canister was closed and disconnected from the gasket. The clogged filters were removed from the gasket as described above and a new filter was placed into the gasket to continue the sample processing. All the filters used to process a single sample were pooled together in the same tube. In a single case, 9 filters were needed to process a single sample (Station SO226-3-7, 0-100 m). These 9 filters have been split into two tubes. The whole process, from the sample recovery to their complete processing took less than one hour. A total of 45 samples have been processed during the

cruise, comprising 30 samples collected from the 5 depth intervals (0-100m, 100-200m, 200-300m, 300-500m and 500-700m) at 6 stations (Table 5.1), 6 samples from a replicated collection of the 0-100 m interval of the same 6 stations and 9 samples collected from the 0-100 m depth interval of the remaining 9 stations. Three additional samples were produced by pooling the plankton residues after picking from all depth intervals at three stations (labelled as “Blank” Table 5.1). These samples were produced to be used as expendable test material.

References

Bé, A.W.H. (1977), An ecological, zoogeographic and taxonomic review of recent planktonic foraminifera. In. Ramsey, A.T.S. (Ed.), Oceanic Micropaleontology, Volume 1, Academic Press, London; pp. 1–100.

Hemleben, C., et al. (1989), Modern planktonic foraminifera. Springer, New York, 363 pp.

Morard, R., et al. (2009), Morphological recognition of cryptic species in the planktonic foraminifer Orbulina universa. *Marine Micropaleontology* **71**, 148-165.

Parker, F.L. (1962), Planktonic foraminifera species in Pacific sediments. *Micropaleontology* **8**, 219–254.

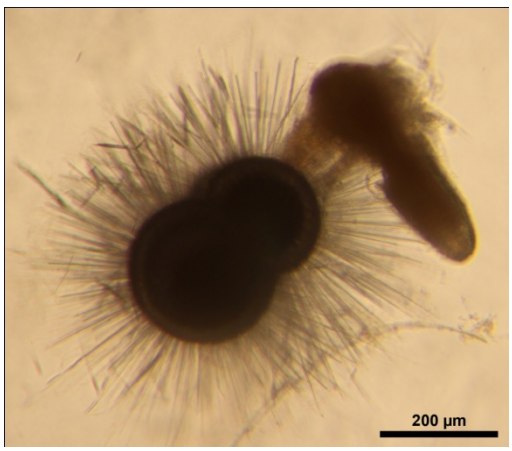
6. Foraminifera Culturing

Selected healthy, adult specimens of several planktonic foraminifer species were placed into cultures, fed and monitored during the cruise. One of the aims of that procedure was to test the possibility of foraminifera culturing under the restricted conditions on a scientific vessel. Furthermore, it enabled the specimens to recover after sampling, allowing observations of spines, cytoplasm and feeding behaviour in these specimens under close-to-natural conditions.

Sarstedt cell-culturing flasks (50 ml) with a semi-permeable cap, filled with sea water filtered using a 0.22 µm filter, were used to harbour one foraminifer specimen each. To stabilize culturing conditions, the flasks were placed in a flow through device, which continuously adapted culturing temperatures to local surface environmental conditions. This device consisted of a plastic tank with an overflow hole at about 2/3 of its height. It was continuously supplied with ambient seawater from the ship's uncontaminated seawater supply. An aluminium basket was partially submerged in the water bath, allowing easy handling of the culturing flasks. The culturing setup was placed in a sink beneath a window, so that lighting conditions followed approximately natural day-night-cycles.

For feeding purposes *Artemia* larvae were cultured in separate flasks, and one larvae was fed to each foraminifer approximately once per day. For that, *Artemia* larvae intended for feeding were transferred into a small Petri dish and single individuals were separated. The larvae were incapacitated with a needle, transferred into the culturing flasks, and manually placed on the spines of the foraminifers with a needle or brush.

Several specimens of planktonic foraminifera could be kept alive in this culturing setup for several days and accepted *Artemia* larvae almost daily (Fig. 6.1). After some days, spinose species rebuilt their spines, and symbionts were observed on the outside of the shell. One specimen of *Hastigerina pelagica* survived for two weeks and reached gametogenesis. Initial signs of that process were the reduction of the bubble capsule, destruction of the terminal chamber and partial resorption of spines (Fig. 6.2). One day later, on 23.3.2013, 12 days after new moon, after an additional feeding, gametes were observed in the cytoplasm of the individual and the specimen was removed from culture and preserved in GITC* buffer for further analyses.



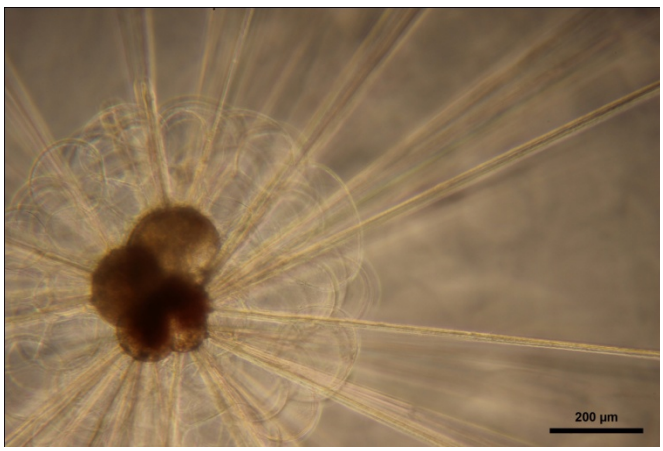


Fig. 6.1 *Globigerinoides trilobus* feeding on *Artemia* (left) and *H. pelagica* with bubble capsule (right).

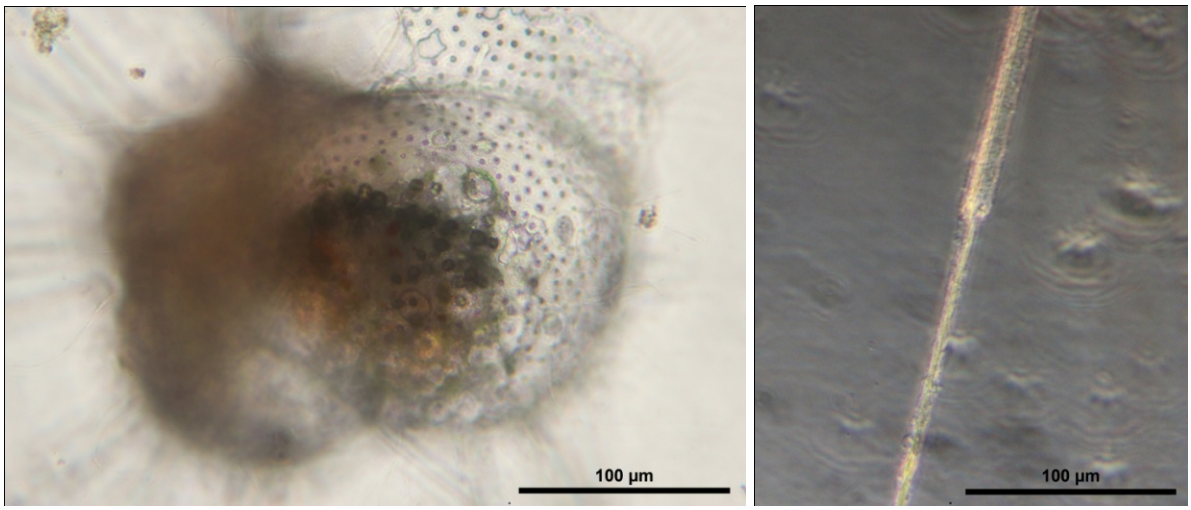


Fig. 6.2 *Hastigerina pelagica* prior to gametogenesis. (left) The cytoplasm has retracted from the last and partly also from the penultimate chamber, the last chamber (top of image) was partly destroyed and (right) some of the spines were resorped. Approximately 12 hours after observing those processes the gametogenesis took place.

Tab. 6.1 List of specimens that were held in culture during the expedition SO226-3.

Species	Station	Sample	Days in culture	Status
Globigerinoides sacculifer	226-3-2	K277_060-040	1	discarded
Globigerinoides ruber	226-3-2	K277_060-040	1	discarded
Globigerina incompta	226-3-2	K277_060-040	1	in buffer
Globigerinoides ruber	226-3-2	K277_060-040	1	in buffer
Globigerinella calida	226-3-3	K279_020-000	4	discarded
Globigerinoides sacculifer	226-3-3	K279_040-020	4	discarded
Globigerinita glutinata	226-3-3	K279_500-300	6	discarded
Globigerinoides ruber	226-3-5	K283_100-080	2	discarded
Globigerinoides sacculifer	226-3-5	K283_020-000	4	discarded
Hastigerina pelagica	226-3-5	K283_020-000	14	in buffer after gametogenesis
Globigerinita glutinata	226-3-5	K283_020-000	2	discarded
Globigerinella siphonifera	226-3-5	K282_200-100	1	discarded
Globigerinoides sacculifer	226-3-9	K292_060-040	8	in buffer

7. Shipboard Results

7.1. Hydrography

The hydrographic data obtained during the cruise (Fig. 7.1.1 and 7.1.2) indicates that all major currents of the western central Pacific have been sampled, including the particularly complex hydrography of the surface and upper intermediate water of the western central Pacific, where the large anticyclonic gyres in the north and south Pacific lead to the development of various surface and subsurface counter-currents. The upper left panel of Figure 7.1.3 gives an overview of the main hydrograhic features encountered during the cruise. Station SO226-3-2 serves as a representative for the cool, saline and well oxygenated waters of the south Pacific encountered between New Zealand and Vanuatu (40° to 20°S). Station SO226-3-6 represents with its subsurface waters the northern arm of the South Equatorial Current (SEC). This saline and warm water originates in the central South Pacific (Reid 1997). Station SO226-3-12 sampled water of the North Equatorial Countercurrent (NECC) with its low salinity surface waters, resulting from high precipitation in this region (Dayem et al. 2007). Further north, at station SO226-3-14, the subsurface salinity maximum of the North Equatorial Current (NEC) has been encountered, which could be identified by lower absolute values than its southern counterpart (Fine et al. 1994).

A closer look at the first five stations (Fig. 7.1.3) reveals a remarkably homogenous water column structure. Station SO226-3-1 appears to be located south of the Tasman front and shows therefore the largest difference in hydrographic structure. The decrease in temperature and salinity for the deepest measurements of stations SO226-3-4 and 5 are due to the influence of submerged Antarctic surface waters flowing northwards east of the ridge extending northwards from New Zealand (Reid 1997).

The hydrographically most interesting section is the central part of the cruise track between 10°S and 10°N (Fig.7.1.3). Stations SO226-3-6 and 7 sample the northern limb of the SEC as indicated by the pronounced subsurface salinity maximum. Station SO226-3-8 at 1.4°S shows a diminishing subsurface salinity maximum and a comparatively high surface salinity. Station SO226-3-9 at 2.6° N is positioned on the northernmost extent of the Equatorial Undercurrent (EUC), which is a subsurface counter-current centred on the Equator at ~200 m depth. The EUC is not clearly distinguishable by its salinity, but the shallow subsurface salinity maximum at station SO226-3-9, which is still caused by SEC water extending over the equator, indicates its presence (Reid 1997). Stations SO226-3-10 to 12 north of 4.6°N sample the low saline and quasi-isothermal surface waters of the NECC. Below 300 m depth the uppermost reaches of the North Pacific oxygen minimum zone can be seen (Fig. 7.1.2) with oxygen concentrations as low as 1.1 ml/L (< 25% oxygen saturation).

Northward of station SO226-3-12 (Fig.7.1.3) we finally encounter true North Pacific waters in form of the NEC. It exhibits a subsurface salinity maximum similar to the SEC, but lower in absolute values due to the overall lower salinity in the North Pacific (Reid 1997). Further to the north the subsurface salinity maximum decreases in amplitude and descends in depth. The upper boundary of the oxygen minimum also descends to 500 m (Stations SO226-3-13 and 14) and 600 m (Stations SO226-3-15 and 16).

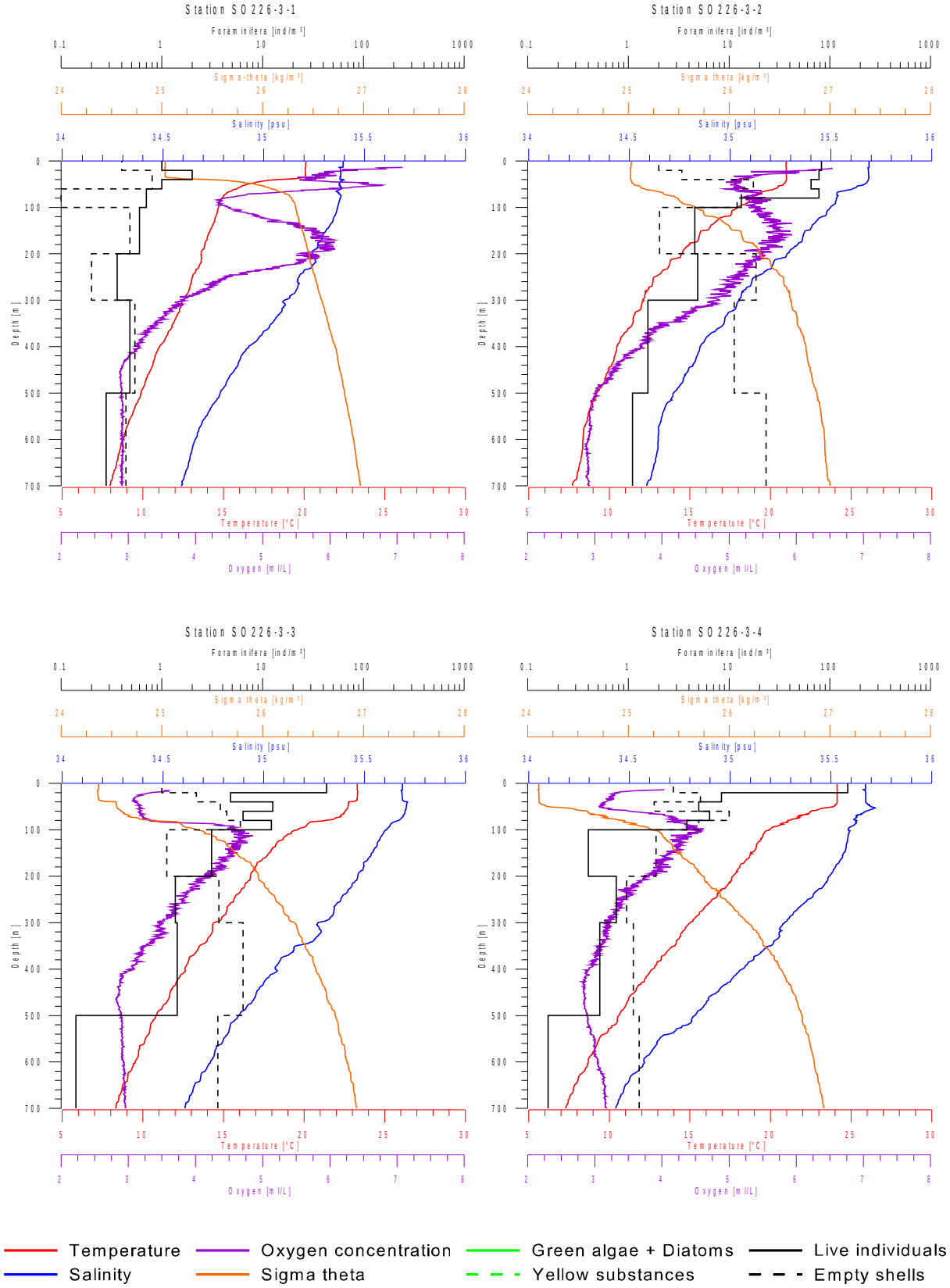


Fig. 7.1.1 Vertical profiles of hydrographic parameters (temperature, salinity, potential density and oxygen concentration) from CTD casts, phytoplankton pigment concentrations (from Station SO226-3-6 onwards) measured by fluorescence probe on CTD-Rosette water samples and the concentration of live specimens and empty shells of planktonic foraminifera for each station of the cruise SO226-3.

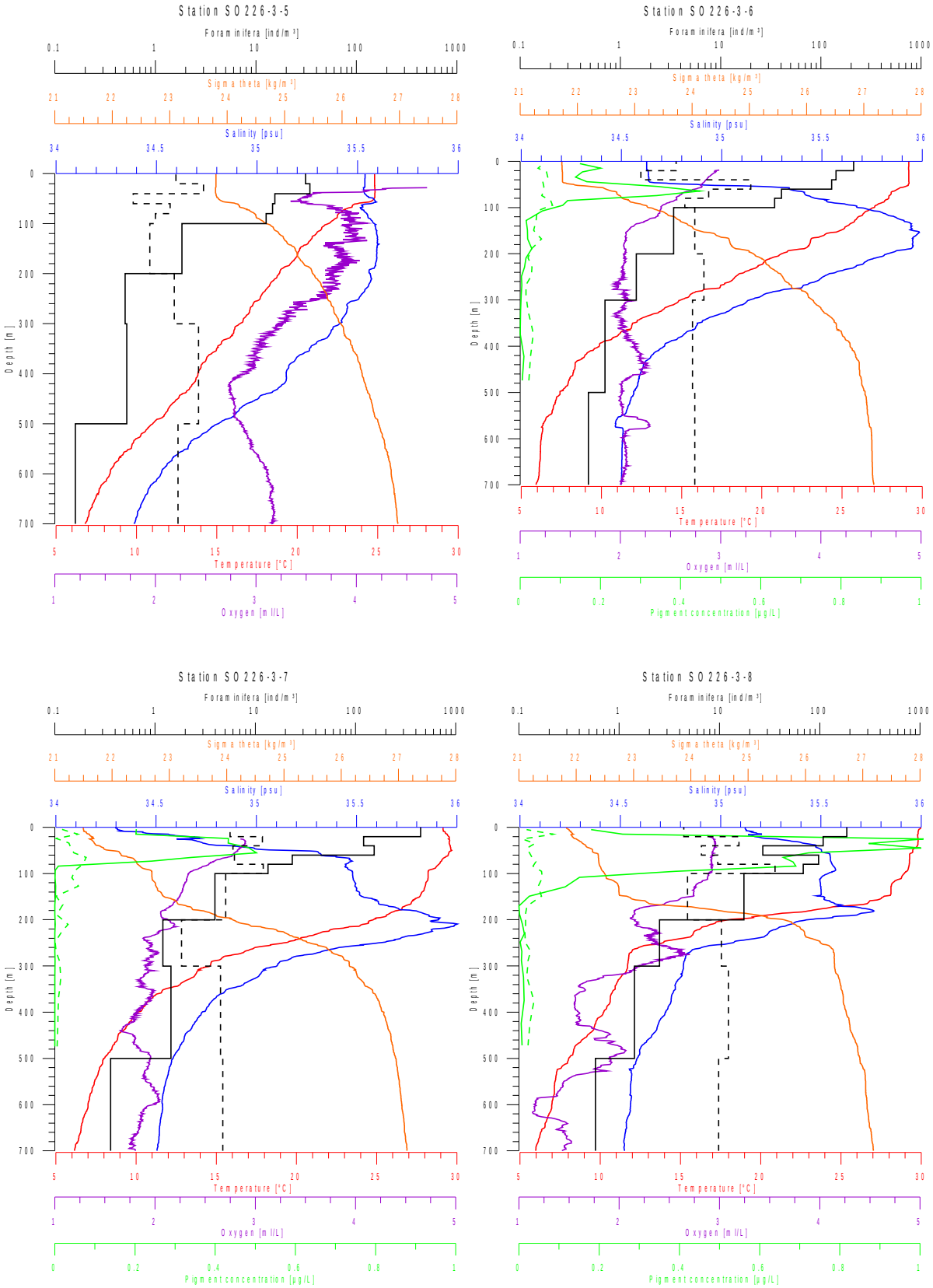


Fig. 7.1.1 (continued) Vertical profiles of hydrographic parameters (temperature, salinity, potential density and oxygen concentration) from CTD casts, phytoplankton pigment concentrations (from Station SO226-3-6 onwards) measured by fluorescence probe on CTD-Rosette water samples and the concentration of live specimens and empty shells of planktonic foraminifera for each station of the cruise SO226-3.

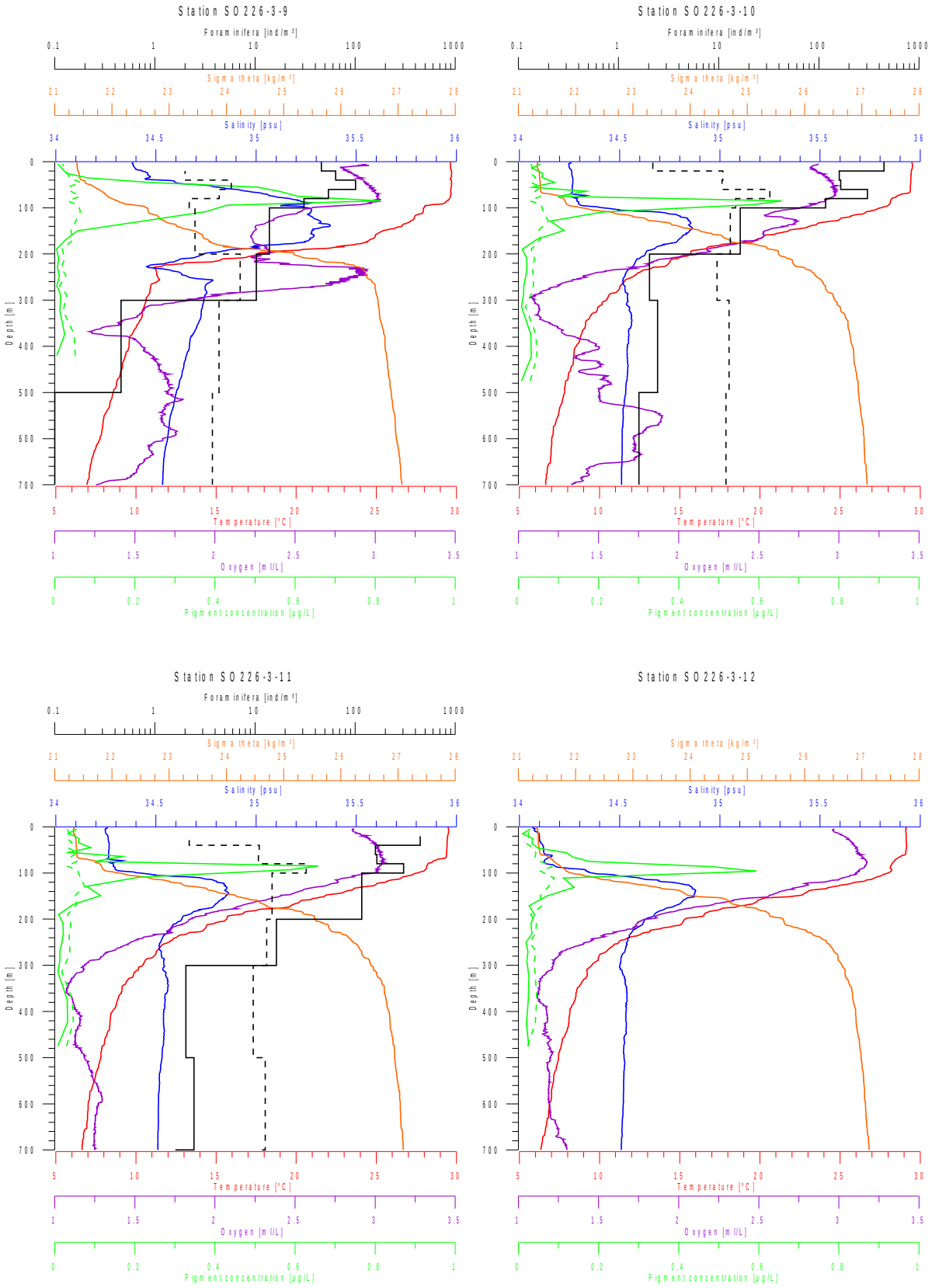


Fig. 7.1.1 (continued) Vertical profiles of hydrographic parameters (temperature, salinity, potential density and oxygen concentration) from CTD casts, phytoplankton pigment concentrations (from Station SO226-3-6 onwards) measured by fluorescence probe on CTD-Rosette water samples and the concentration of live specimens and empty shells of planktonic foraminifera for each station of the cruise SO226-3.

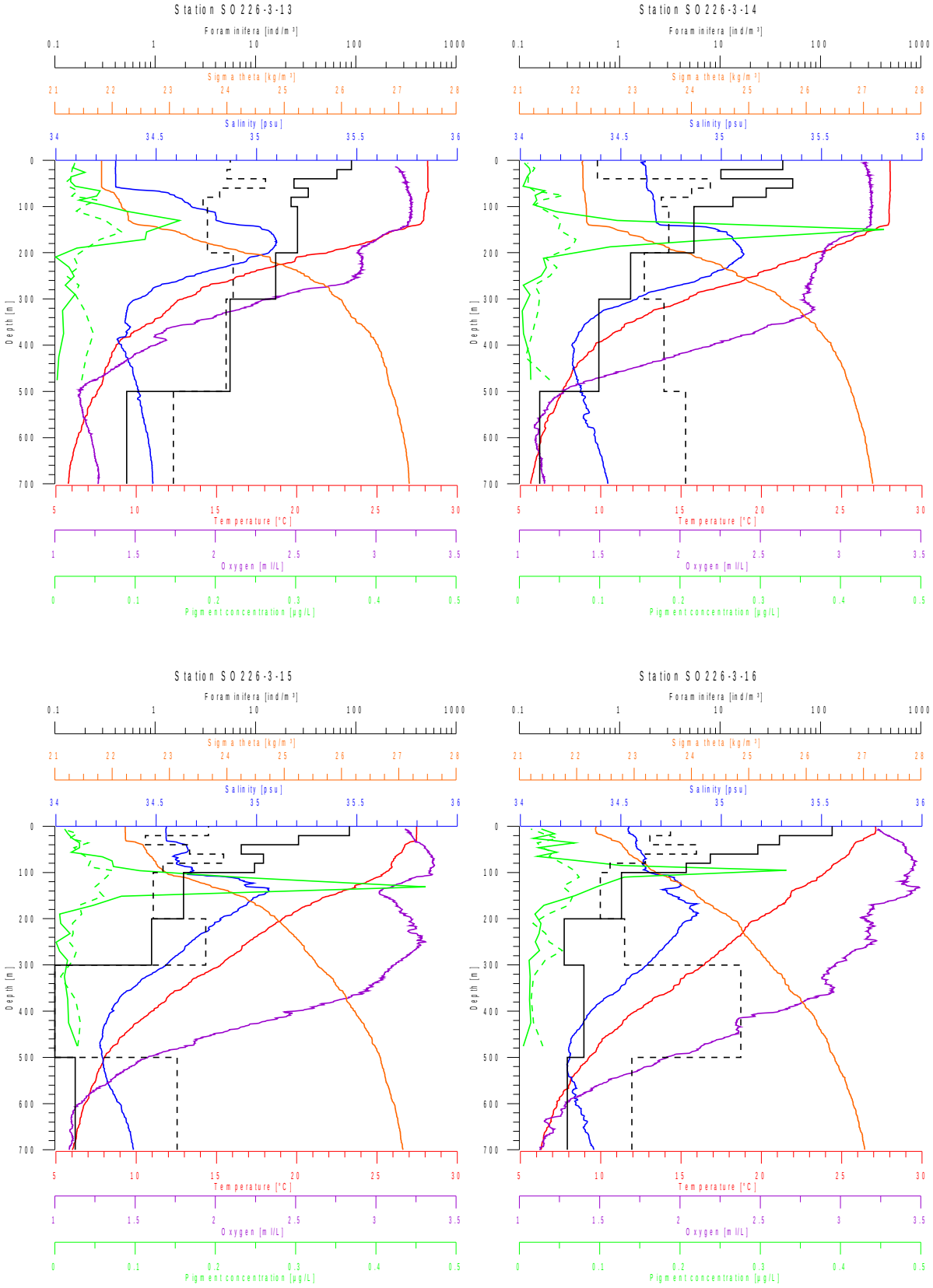


Fig. 7.1.1 (continued) Vertical profiles of hydrographic parameters (temperature, salinity, potential density and oxygen concentration) from CTD casts, phytoplankton pigment concentrations (from Station SO226-3-6 onwards) measured by fluorescence probe on CTD-Rosette water samples and the concentration of live specimens and empty shells of planktonic foraminifera for each station of the cruise SO226-3.

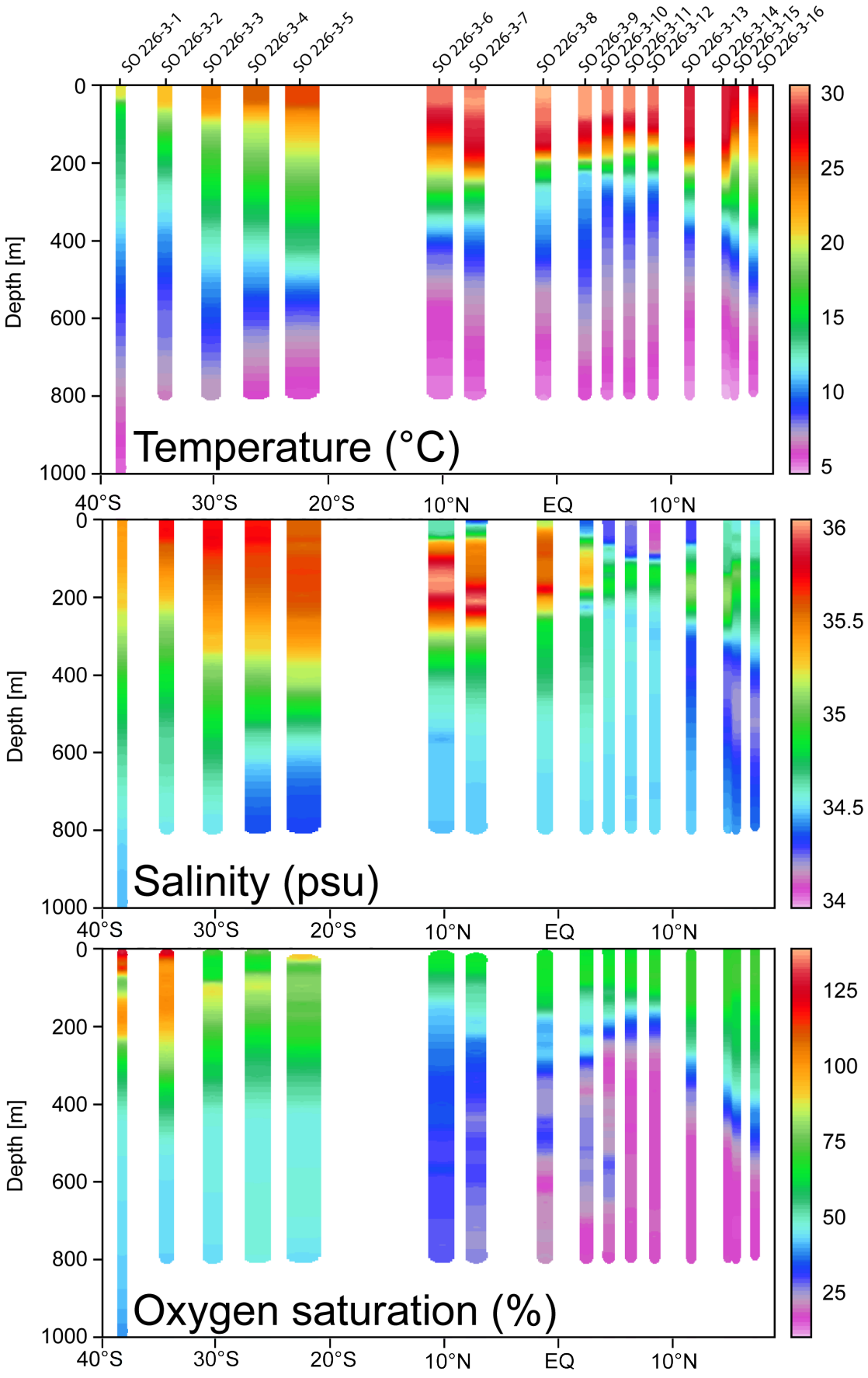


Fig. 7.1.2 Summary of vertical profiles of temperature, salinity and oxygen saturation throughout the upper 1000 m of the water column at all 16 CTD stations of SO226-3 ordered by latitude.

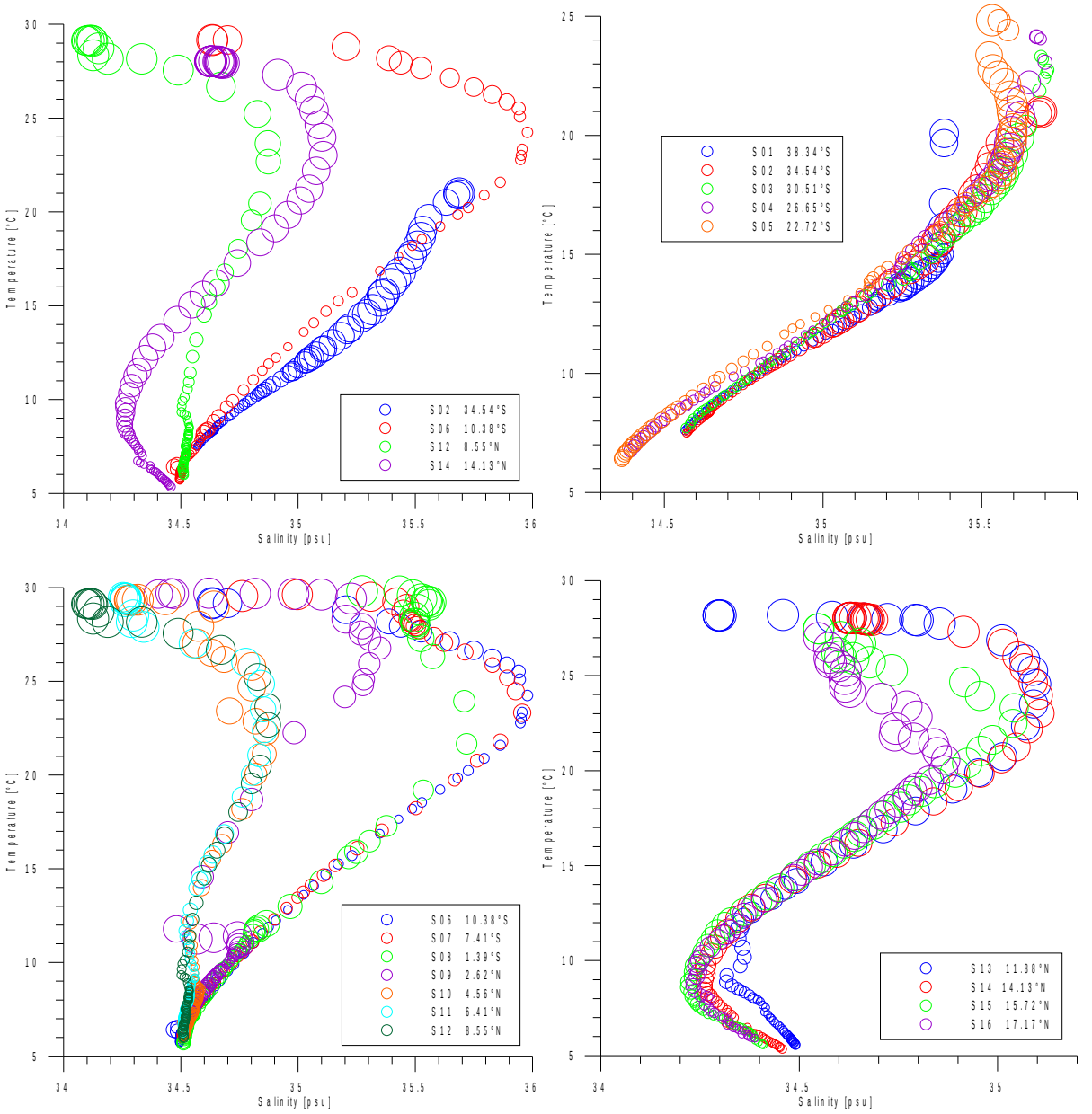


Fig. 7.1.3 Temperature/Salinity plots of the CTD data from cruise SO226-3. CTD data are binned to 10 m intervals. Size of symbols indicates oxygen concentration relative to individual station minimum and maximum.

7.2. Phytoplankton distribution

Based on satellite observations (Fig. 4.2), stations SO226-3-1 and 2 show elevated chlorophyll *a* concentrations, probably due to their proximity to New Zealand coastal waters combined with elevated nutrient concentrations in the Tasman front (Station SO226-3-2 only). The South Equatorial Current supplies nutrient poor waters to stations SO226-3-3 to 5, which show standard open ocean chlorophyll *a* concentrations. From Station SO226-3-6 onwards, direct measurements of phytoplankton pigments in the water column are available. Stations SO226-3-6 and 7 are situated in an area of increased productivity east of the Solomon Islands. Messie and Radenac (2006) propose wind-induced Ekman pumping as the reason (as

indicated by compression of the isotherms). The following station SO226-3-8, which showed the highest measured pigment concentrations during the cruise (1 µg/l (green algae + diatoms) centred at ~30m depth, Fig. 7.2.1) lies in the westernmost reaches of the Equatorial Upwelling zone, where upwelling of nutrient rich water is induced by the equatorial divergence of winds and consequently currents. Maximum pigment concentrations of stations SO226-3-9 to 12 are lower (0.8 to 0.6 µg/l) and lie at ~70 m depth at the base of the pycnocline in form of a deep chlorophyll maximum. Since satellite sensors do not reach that deep, the stations are shown with standard low oceanic productivity in the composite satellite image (Fig. 4.2). Stations SO226-3-13 to 16 are situated in the ultra-low productivity oceanic environment (ocean deserts). Maximum pigment concentration do not surpass 0.5 µg/l and the deep chlorophyll maximum descends to 100 m and deeper.

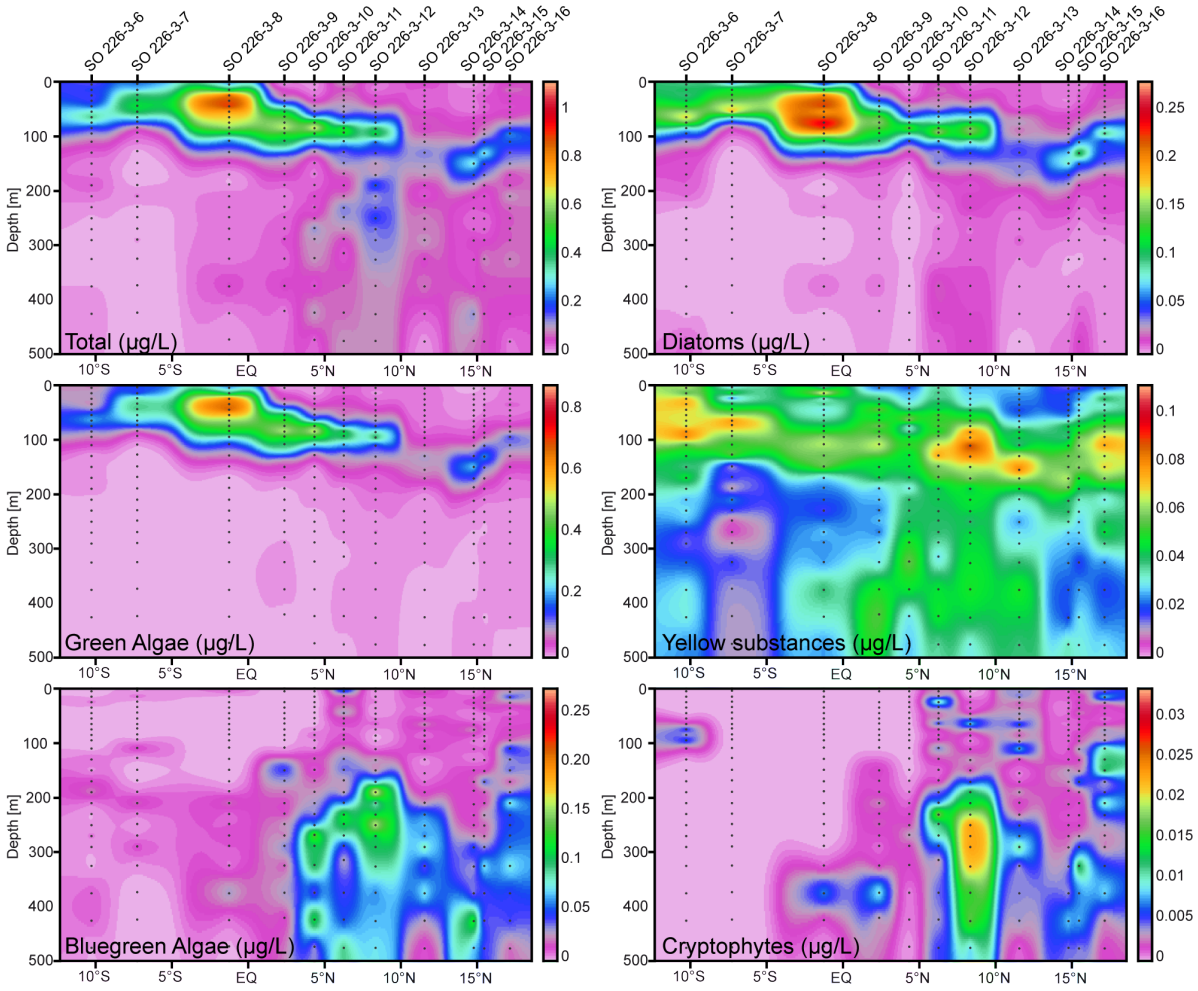


Fig. 7.2.1 Summary of vertical profiles of phytoplankton pigment concentration throughout the upper 500 m of the water column at the 10 CTD stations of SO226-3, where these measurements have been carried out, ordered after latitude. Black dots indicate the midpoint depths positions of the actual samples. Raw data are presented in Fig. 7.1.1.

The phytoplankton pigment distribution indicates a dominance of green algae at all stations, followed by diatoms (Fig. 7.2.1). Yellow substances reach maximum concentrations at the depth around 100 m and appear to track the lower boundary of the chlorophyll maximum. Their concentration does not decay with depth, confirming that they correspond to pigment decay products. Beginning with station SO226-3-10, a consistent signal of elevated concentrations of bluegreen algae at depth below

150 m is observed (Fig. 7.2.1); its meaning is not clear. The concentrations of cryptophytes are low at all stations and depths, too close to the detection limit to permit meaningful interpretations, other than they generally appear to track the signal of the bluegreen algae.

7.3. Distribution and diversity of planktonic foraminifera

A total of 44788 specimens of planktonic foraminifera have been collected throughout the cruise. Of these, 32077 (72%) possessed cytoplasm and 13426 (18%) appeared empty. Plots of the concentration of planktonic foraminifera at each station are given in Figure 7.1.1. The highest concentration of live specimens was within the top 100 m of the water column, harbouring on average 84% of the live assemblage. At all stations, the highest concentrations of live specimen have been observed at the surface (0-60 m); at all but four stations in the 0-20 m interval. The average habitat depth was deeper at stations taken in the equatorial region (Fig. 7.3.1); at Station 13 more than half of the live assemblage dwelled below 100 m.

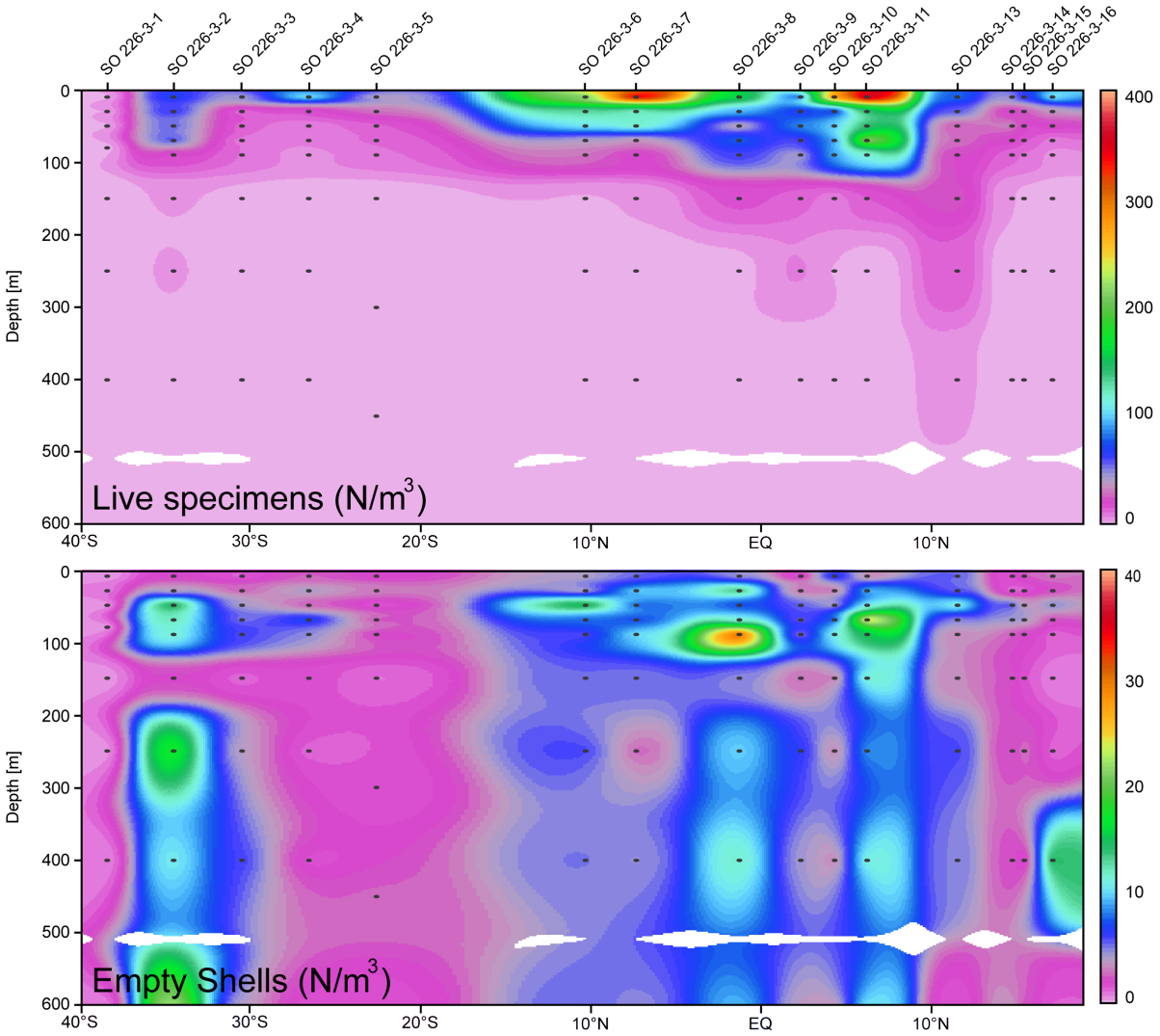


Fig. 7.3.1 Summary of vertical profiles of the concentration of live specimens and empty shells of planktonic foraminifera in the fraction >0.1 mm throughout the upper 600 m of the water column at the 15 multinet stations of SO226-3, ordered by latitude. Black dots indicate the midpoint depths of the actual samples. Raw data are presented in Fig. 7.1.1.

The highest concentrations of live specimens were encountered in the surface layer of stations SO226-3-7, 10 and 11, all located between 10°S and 10°N (Fig. 7.3.1). The maximum concentrations in the top 20 m of the water column exceeded 400 specimens/m^3. Besides Station SO226-3-1, where the surface haul was affected by a net tear, the lowest concentration of live specimens was observed at Stations SO226-3-3,4,5,14,15, all located in the subtropical gyre regions. Stations 13-15 showed the lowest total phytoplankton concentration of all stations where this variable has been measured (Fig. 7.3.2), but beyond this observation, there does not appear to be any obvious relationship between the abundance of foraminifera and the concentration of the phytoplankton. Similarly, there does not seem to be any relationship between abundance of foraminifera, neither in total nor at any particular depth, with the lunar cycle. The new moon occurred during the cruise around the position of Station SO226-3-6 and full moon occurred two days after Station SO226-3-16, which both show neither an anomalously high or low concentration nor an unusual vertical distribution profile (Figs. 7.3.1 and 7.3.2).

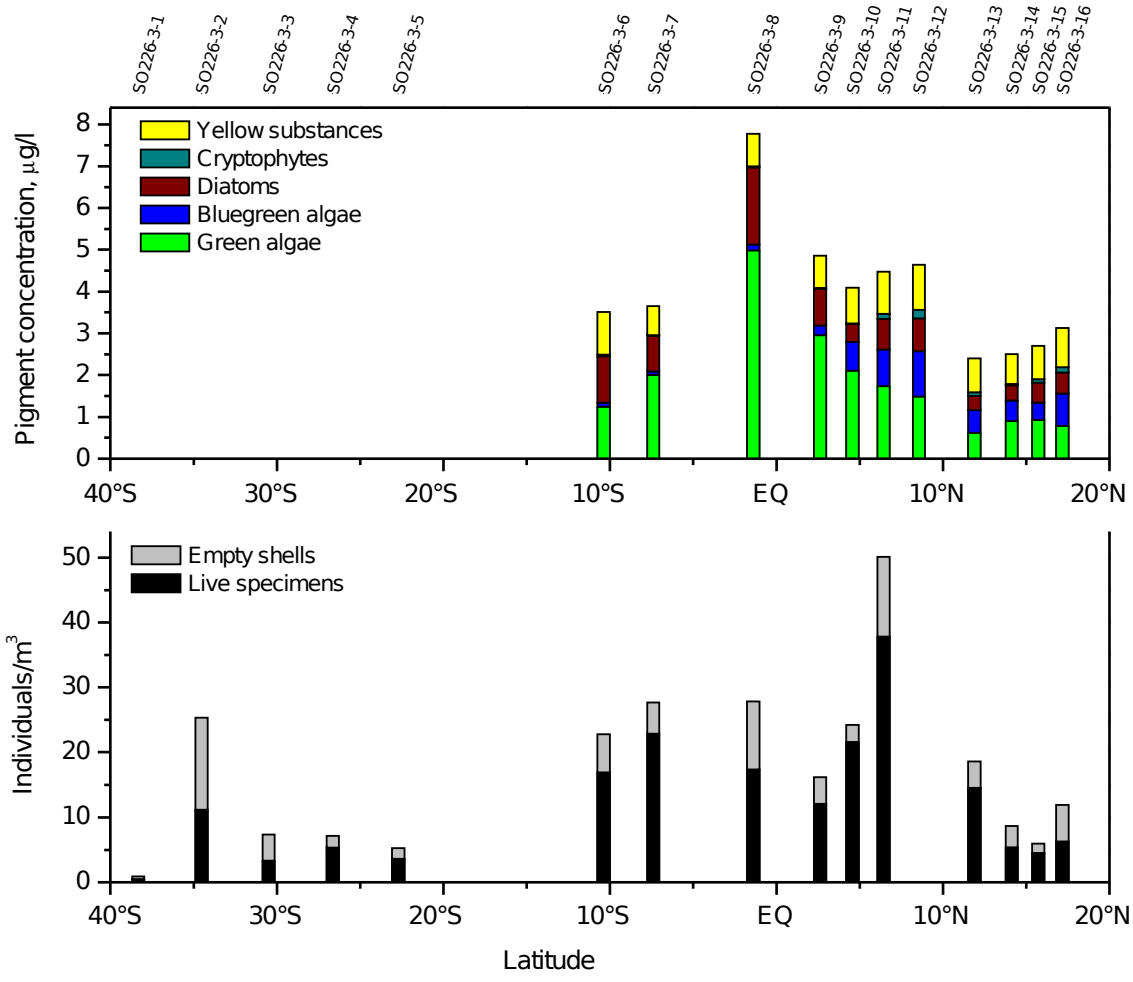


Fig. 7.3.2 Total amount of phytoplankton pigments and average concentration of planktonic foraminifera shells at all stations of the cruise SO226-3 ordered by latitude.

The concentration of empty shells of planktonic foraminifera was on average lower by a factor of three than that of live specimens. Maxima in the concentration of empty shells occurred at all stations below the surface, typically between 40 and 100 m. Unlike the distribution of live specimens, which was tightly linked to the surface, the concentration of empty shells was more even across the sampled depth intervals,

reflecting a steady rain of dead shells to the sea floor (Fig. 7.3.1). The distribution of the total flux of empty shells along the cruise track was characterised by the presence of three “deposition events” recorded at Stations SO226-3-2, 8 and 11 (Fig. 7.3.1). These events do not appear to be linked to synchronised reproduction, as the maxima occurred 5 days before and 3 and 7 days after the new moon, respectively. Rather, we assume that these events reflect regional historical contingencies.

Whereas the concentration of live specimens and empty shells of planktonic foraminifera are available for all stations at all depth levels, the occurrence and abundance of individual species has been determined only in a subset of these samples. Because of the large numbers of specimens in certain samples, combined with the adverse conditions on board, taxonomic census counts have been produced only for 60 out of 134 samples (one sample being one depth level at a station). However, these 60 samples only account for 7726 of a total of 44788 encountered specimens and only three stations have been counted at all depth levels. Therefore, it is import to stress that the available counts are in no way representative of assemblage composition throughout the entire cruise. On the other hand, the available counts can be used to obtain a first approximation on the species present, their relative rank and distribution with depth.

Among the counted samples, 38 species have been encountered at least once. In addition, specimens of *Globigerinoides sacculifer* both with and without the final sac-like chamber have been counted separately, although it now appears that these represent a single biological species (André et al., 2013). The distinction between *Globigerinoides ruber* and *Globigerinoides elongatus* has been made wherever possible, although we note, like Aurahs et al. (2011) that the identification of the latter species is difficult among juvenile specimens. Of the 38 species, only one could not be assigned to an existing taxonomic category. This small (<0.15 mm), thin-walled, apparently microperforate form, with four equally-sized chambers in the last whorl and an umbilical-extraumbilical aperture, occurred abundantly at subtropical stations (St. 3, 14 and 15). It was found both empty and with cytoplasm, which was consistently bright green in colour. In the absence of a more secure determination, this species is referred to as *Tenuitella*? sp. Juvenile specimens in the size range close to 0.1 mm have not been further differentiated. These were in all cases clearly distinguishable from adult specimens of small species and most likely combine individuals of *Globigerinoides ruber*, *Globigerinoides sacculifer* and *Globigerina bulloides*.

A total of 40 specimens of the species *Globorotalia theyeri* have been encountered. These specimens could be easily distinguished from *G. crassaformis* by a more lobate axial outline and a thinner, biconvex shell with a more acute periphery. The keel was not seen and the shells were not spiroconvex, distinguishing this species clearly from *G. hirsuta*. *G. scitula* was observed frequently in our samples; its thin, small, translucent shells were also distinct from *G. theyeri*, leaving us to conclude that the species concept of this taxon is well founded and that this species represents a rare but conspicuous element of the subtropical Pacific thermocline fauna. Assignment of a few specimens to *Tenuitella fleisheri*, *Tenuitella parkerae* and *Berggrenia pumilio* will require SEM confirmation. Seven specimens of benthic foraminifera have been identified in the counted samples, including one specimen of the semi-planktonic *Cymbaloporetta* and two specimens of the genus *Amphistegina*.

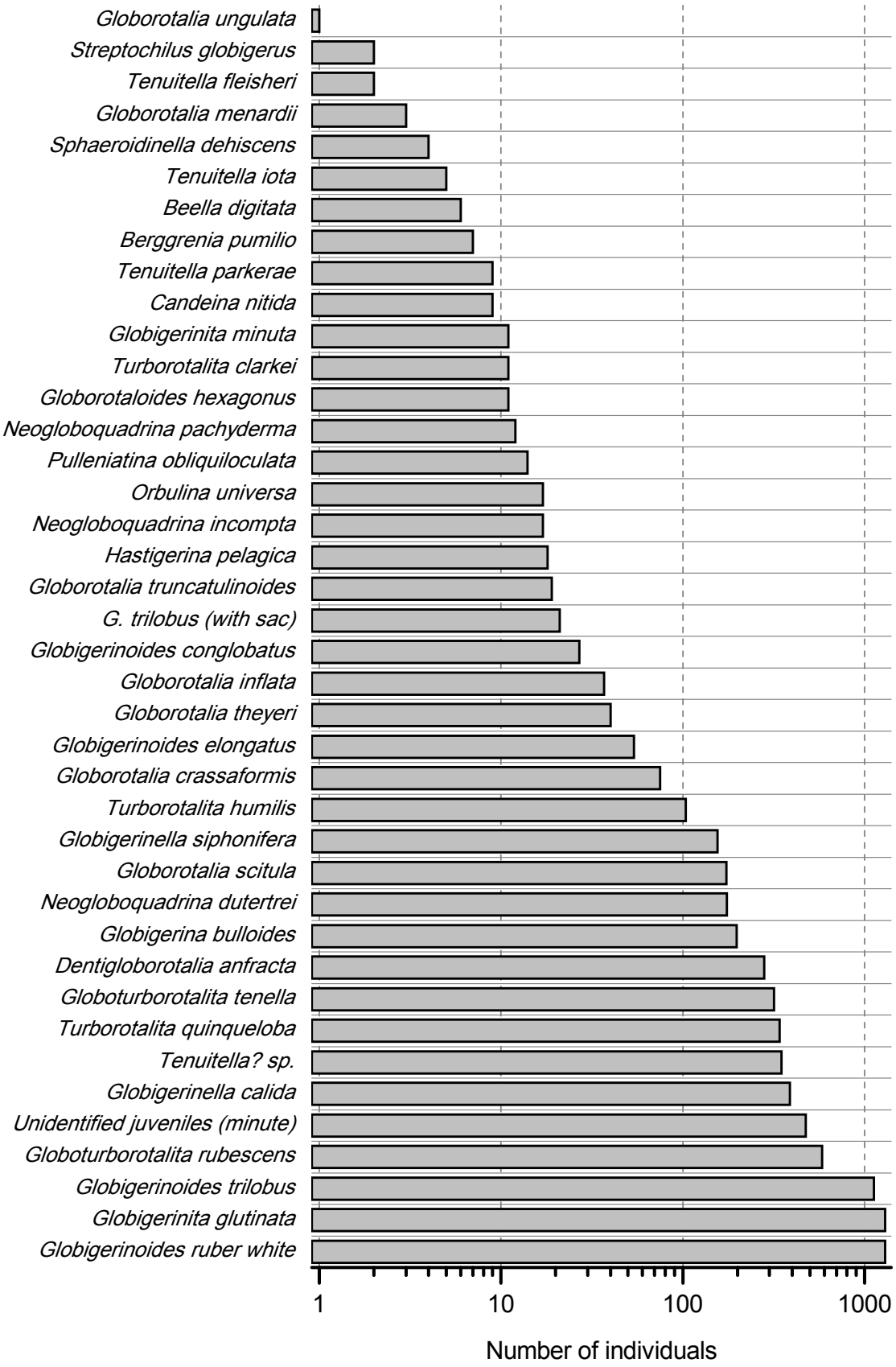


Fig. 7.3.3 Distribution of species abundances in the total assemblage (living + empty specimens) pooled across all 60 samples where census counts have been carried out on board SO226-3.

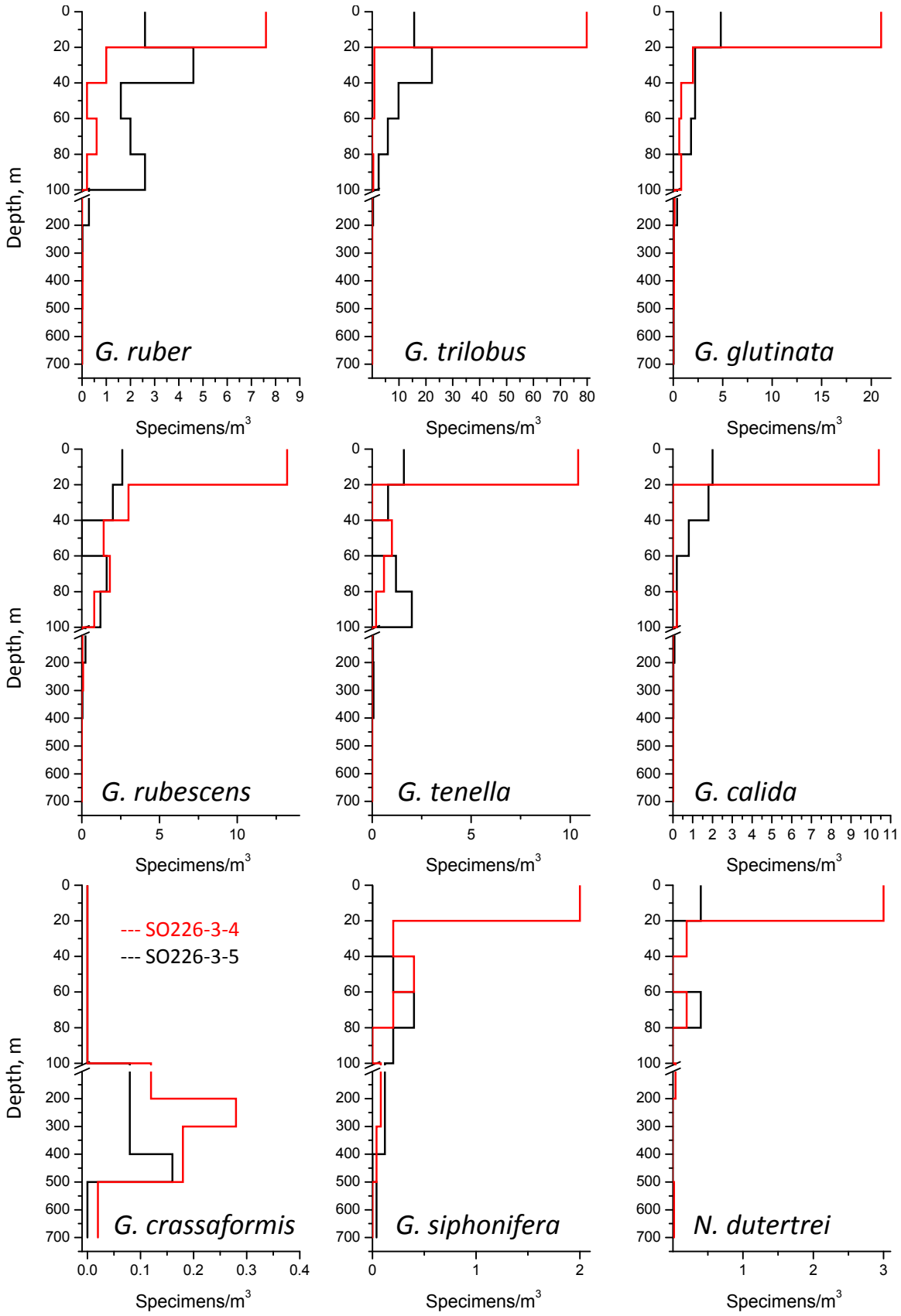


Fig. 7.3.4 Vertical distribution of live specimens of nine abundant species at two stations of SO226-3 in the subtropical waters between New Zealand and New Caledonia.

The species allocation graph indicates a lognormal distribution of species abundances (Fig. 7.3.3). The species *Globigerinoides ruber*, *Globigerinita glutinata*, *Globigerinoides trilobus* and *Globoturborotalita rubescens* together make up more than half of the assemblage. The 25 least abundant species combined comprise less than 5% to the total census. Of note are the high abundance of *Globigerinella calida* and the low abundance of *Orbulina*, *Pulleniatina* and *Hastigerina*.

Complete counts at Stations SO226-3-3 and 4 in the subtropical waters between New Zealand and New Caledonia allow a first evaluation of the vertical habitat of several species (Fig. 7.3.4). The subsurface habitat of *Globorotalia crassaformis* contrasts with the observed strong surface affinity of *Globigerinita glutinata, Globigerinella calida* and *Globoturborotalita rubescens*. *Globigerinoides ruber* and *Globoturborotalita tenella* are distributed more evenly throughout the top 100 m of the water column at Station SO226-3-3, whereas life specimens of *Globigerinella siphonifera* are also found below 100 m.

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8. Station List and Data Availability

Station data, as well as all processed data from CTD casts and fluorospectrometer measurements are available on the PANGAEA portal: http://www.pangaea.de/

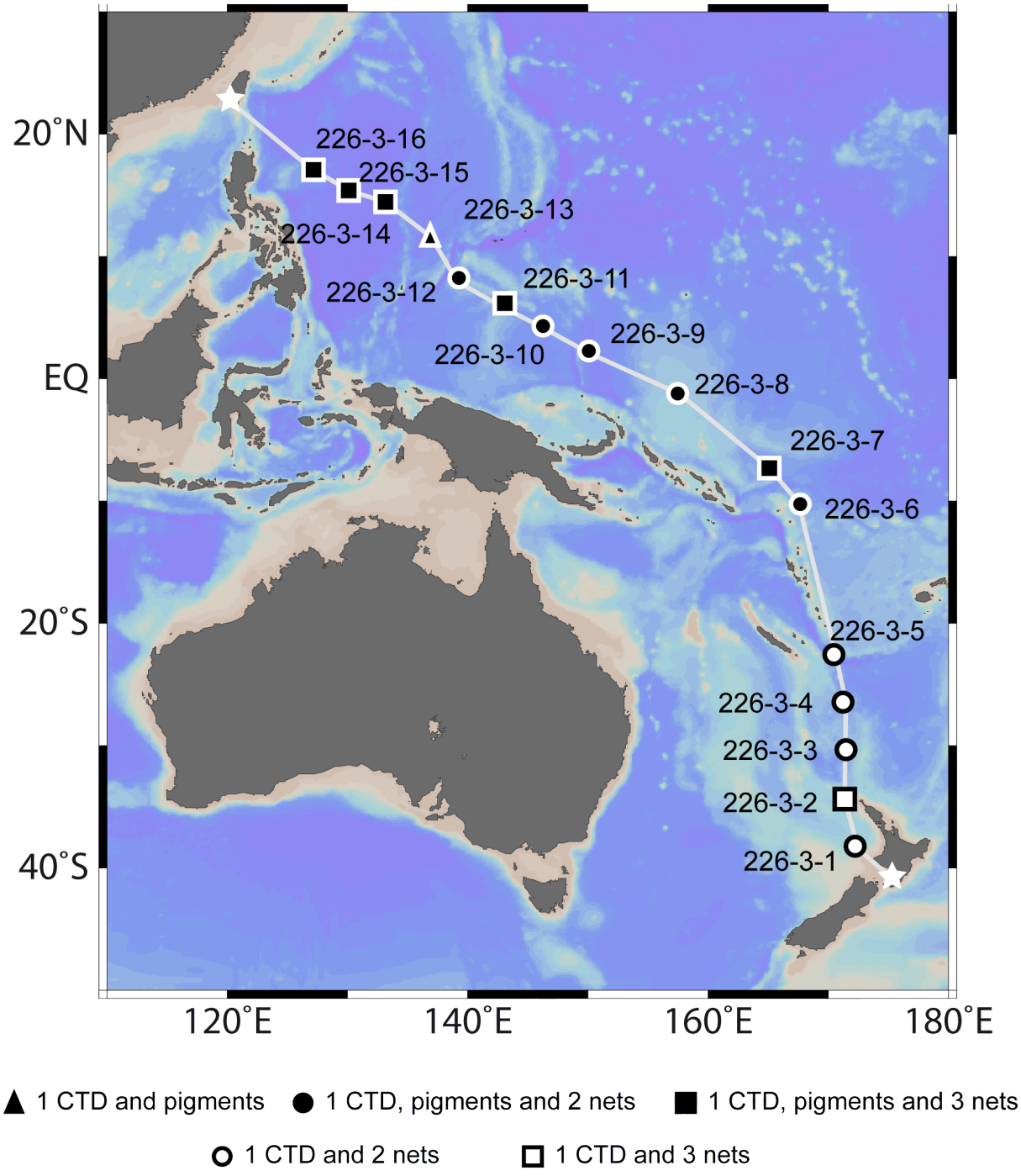

Fig. 8.1 Cruise track with the position of all stations (see Table 8.1) of SO226-3 and an indication of the sampling equipment deployed at each station.

Tab. 8.1 Station list of SO226-3 indicating sampling equipment deployed at each station. WD refers to water depth, PI refers to the number of depth intervals analysed for pigment concentration.

Ship Station Name	Running Station Name	Date [UTC]	Start Time [UTC]	Lat. [deg]	Long. [deg]	WD [m]	CTD Profile	PI	700m Net	100m Net
SO226/107	226-3-1	04/03/13	19:10	-38.340	172.465	1324	1000 m	-	1	1
SO226/108	226-3-2	05/03/13	19:02	-34.536	171.587	1204	800 m	-	2	1
SO226/109	226-3-3	06/03/13	20:02	-30.508	171.906	3235	800 m	-	1	1
SO226/110	226-3-4	07/03/13	20:01	-26.654	171.384	3894	800 m	-	1	1
SO226/111	226-3-5	08/03/13	20:03	-22.719	170.918	4378	800 m	-	1	1
SO226/112	226-3-6	11/03/13	21:00	-10.381	167.125	2378	800 m	24	1	1
SO226/113	226-3-7	12/03/13	21:00	-7.409	165.274	3511	800 m	24	2	1
SO226/114	226-3-8	14/03/13	23:31	-1.388	157.925	1916	800 m	24	1	1
SO226/115	226-3-9	16/03/13	22:00	2.616	150.260	5057	800 m	22	1	1
SO226/116	226-3-10	17/03/13	22:00	4.561	146.540	4327	800 m	24	1	1
SO226/117	226-3-11	18/03/13	22:00	6.414	143.024	2313	800 m	24	2	1
SO226/118	226-3-12	20/03/13	00:22	8.549	139.597	4430	800 m	24	-	-
SO226/119	226-3-13	20/03/13	22:00	11.879	137.143	4982	800 m	24	1	1
SO226/120	226-3-14	21/03/13	23:00	14.126	133.479	5420	800 m	24	2	1
SO226/121	226-3-15	22/03/13	23:00	15.716	130.290	5207	800 m	24	2	1
SO226/122	226-3-16	23/03/13	23:00	17.171	127.370	5481	800 m	24	2	1

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